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REVIEW

Molecular methods for rapid detection and identification of foodborne pathogenic bacteria

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Abstract

Foodborne pathogenic bacteria are one of the main factors causing food safety issues. The rapid and accurate detection of pathogenic bacteria using molecular techniques is an effective and powerful strategy for preventing and controlling outbreaks of foodborne diseases, thereby ensuring food safety. This article summarizes the rapid and efficient molecular diagnostic techniques for detecting pathogenic bacteria, including polymerase chain reaction and its derivatives, isothermal amplification, DNA hybridization, genomic sequencing, and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-associated (CRISPR/Cas)-based detection technique. Through a comparative analysis of the technical principles, advantages, and potential limitations of these diagnostic methods, as well as an outlook on the future development directions for molecular biological detection technology, which will provide a valuable reference for developing more accurate, convenient, and sensitive methods for foodborne pathogens detection, and will help better address the challenges posed by foodborne diseases, thereby ensuring public health and safety.

Keywords Pathogenic bacteria · Foodborne diseases · Molecular diagnostic technology · Rapid detection · Food safety

Introduction

The threat of foodborne pathogens to food safety risks has seriously endangered global human health and economic stability. The World Health Organization estimates that there are approximately 600 million cases of global foodborne illnesses and 420,000 cases of deaths each year (Shrivastava et al. 2020). In most of these cases, common foodborne pathogenic bacteria that threaten human health and serious illness, including *Bacillus cereus*, *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Clostridium* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Shigella* spp., are responsible for the largest number of foodborne disease outbreaks worldwide. These pathogens usually cause recurrent intestinal inflammation, diarrhea, vomiting, fever, chronic kidney disease, blindness, and even death (Ghosh et al. 2025; Kabiraz et al. 2023; Sarno et al. 2021). Hence, they have been listed for outbreaks of foodborne illness by standard organizations, such as the European Food Safety Authority (Boukharouba 2022).

Current food safety practices are gradually being optimized and improved under strict regulatory measures, but there is an urgent need for advanced methods to quickly and accurately detect pathogens in food in the face of increasingly complex food safety challenges. Consequently, multiple methods have been developed to detect foodborne pathogens in food and prevent and control outbreaks of foodborne diseases (Gao et al. 2024). Traditional foodborne pathogenic bacteria detection and identification methods are limited automation, time-consuming, low detection rates, labor-intensive, and are insufficient to meet the needs of foodborne disease outbreak detection. Recently developed rapid detection technologies mainly include molecular methods and biosensors. Compared with traditional methods, the advantages of biosensors are fast speed, lower cost,

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easy operation, and less workload. However, the results of biosensors are not very reliable, potentially necessitating the development of food-specific sensors, analytical tools, or customized sampling methodologies for different types of food samples (Kabiraz et al. 2023; Panwar et al. 2022), which to some extent limits the ease of its widespread application. The molecular detection technology developed in recent decades is generally more sensitive, more specific, more time-saving, labor-saving, and more reliable than the conventional culture methods, and it has brought major changes to the fast detection technology of foodborne pathogens (Hadi et al. 2023; Mao et al. 2023). Moreover, it has also promoted the transformation of the detection of pathogenic bacteria from the conventional characteristics of morphology, physiology, and ecology to the molecular biology level (Boukharouba 2022; Ndraha et al. 2023). As a result, various quantitative molecular methods have been developed that are available for the foodborne pathogenic bacteria detection (Elbehiry et al. 2023).

This paper reviews the technical principles, advantages, and potential limitations of these existing molecular diagnostic methods, including polymerase chain reaction (PCR) and its derivatives, isothermal amplification, DNA hybridization, genomic sequencing as well as CRISPR/Cas-based detection techniques. And the possible development direction of molecular biological detection in the future is prospected.

PCR

Conventional PCR

Conventional PCR is a technique that uses a specific pair of primers and a DNA polymerase with high thermal stability to specifically amplify the target DNA from tested microorganisms. Since PCR was first used to detect foodborne pathogens *Salmonella typhimurium* from 100 serotypes with a detection rate of 99.4% by amplifying the nucleotide sequences of the *invA* gene (Rahn et al. 1992), it has become very mature and is currently one of the most effective molecular techniques for foodborne pathogens detection, which can detect various foodborne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157: H7, *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella Typhimurium*, and *Shigella* present in food by detecting specific target DNA sequences (Abdelshafy et al. 2025; Aladhadh 2023; Vidyadharani et al. 2021). While pathogen pre-enrichment can improve the detection sensitivity, timely results are not always available. Recent studies have developed more efficient detection methods by the combination of PCR with other technologies such as nanomaterial-based, and CRISPR/Cas-based technologies to enhance the performance of conventional PCR for detecting pathogens in food samples, for example, when coupled with polydopamine nanospheres, it significantly enhanced amplification efficiency and sensitivity, achieving a detection limit of 6.7×10^4 CFU/mL without pre-enrichment, which is far lower than that of conventional PCR (Xia et al. 2022). Although conventional PCR can only detect a specific DNA sequence at a time, its specificity may be inferior to some traditional culture methods due to genetic similarities among microorganisms, increasing the probability of false positives (Abdelshafy et al. 2025). Moreover, PCR cannot distinguish between living and non-living cells and also requires proper procedures to achieve optimal pure DNA concentrations (Nassarawa et al. 2022). Hence, many PCR methods such as real-time quantitative PCR (qPCR), multiplex PCR, multiplex qPCR, and digital PCR (dPCR) have been derived (as listed in Table 1) and are widely used in the detection and identification of foodborne pathogens.

Multiplex PCR

Multiplex PCR was derived from conventional PCR, which is used to amplify multiple target DNA sequences simultaneously by adding multiple primers of different genes of a pathogenic microorganism or different genes of multiplex pathogens into a single reaction, resulting in the simultaneous detection of multiplex target pathogenic microorganisms from food samples and the typing and identification of some pathogenic bacteria (Chen et al. 2023). It reported that multiplex PCR technology was successfully established by using the species-specific primers for the detection of key bacterial foodborne pathogens, such as *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Staphylococcus aureus* (Aladhadh 2023; Tao et al. 2020). It is widely employed for the simultaneous detection of up to three or nine different foodborne pathogens with good detection limits (Moppert et al. 2025). These results indicated that multiplex PCR is a promising technique for the rapid detection of foodborne pathogenic bacteria and can be used for routine surveillance and risk assessment of food sources. However, due to dimers interference of primer, low amplification efficiency, and the inability to separate amplified products via gel electrophoresis, multiplex PCR still has limitations in evaluating results (Cao et al. 2024). Therefore, primer design is critical for enhancing multiplex PCR efficiency, necessitating evaluation of the potential inter-primer correlations used in different PCRs that may affect amplification performance in different PCR.

Table 1 Characteristic and applications of various types of PCR methods for the detection of foodborne pathogens

Methods	Number of Primers	Enzyme	OT (°C)	Reaction Time (min)	Target Template	Detected Pathogens	Limit of detection	Detected foods	Assay time	Reference
PCR	2	DNA polymerase	50–94	50–120	DNA	*V. parahaemolyticus*; *L. monocytogenes*	50 CFU/g; 4.4×10^4 CFU /mL	Codfish; Milk	2–4 h; NR	(Zeng et al. 2020; Prasad et al. 2023)
Multiplex PCR	multiple	DNA polymerase	50–94	50–120	DNA	*L. monocytogenes*; *V. cholerae*, *S. flexneri*	1.8×10^0-1.8×10^3 CFU/10 g; 10^2 CFU/mL	Pathogenic bacteria suspension	10–16 h; NR	(Feng et al. 2020; Tao et al. 2020)
qPCR	2	DNA polymerase	50–94	50–120	RNA, DNA	*E. coli* O157:H7; *B. cenocepacia*	10–10^2 CFU/mL; 260 cells per sample	Milk, ground beef, and cabbage; Fresh vegetables	3 h; NR	(Park et al. 2020; Liu et al. 2023)
Multiplex qPCR	multiple	DNA polymerase	50–94	50–120	DNA	*L. monocytogenes*, *C. sakazakii*, *S. aureus*, *and Salmonella* spp.; *L. monocytogenes*, *Salmonella* spp., *and E. coli* O26:H11	10^2 CFU/mL; 1 CFU/25 g	Milk; Pork, beef, chicken, mung bean sprout	7 h; 20 h	(Qin et al. 2020; Bundidamorn et al. 2021)
dPCR	2	A digestive enzyme or exonuclease	50–94	50–120	RNA, DNA	*S. Thompson*; *V. parahaemolyticus*	2 CFU/mL; 39 CFU/mL	spiked egg samples Bacterial suspension cultured in tryptic soy broth	NR; 25 min	(Kim et al. 2023; Yang et al. 2020)

OT: operating temperature; NR: not reported

qPCR

qPCR is the process of adding fluorescence-labeled probes or fluorescent chemicals into the PCR system, and monitoring the accumulation of fluorescence signals during the PCR amplification process in real-time through instruments. The fluoresce level can be used to quantitatively analyze the concentration of pathogenic organisms present in the tested samples by standard curve (Zhou et al. 2022).

The fluorescent chemicals and fluorescence-labeled probes commonly used for qPCR are SYBR Green I, TaqMan™, and LightCycler™ probes, respectively (Srinivasa et al. 2023). The SYBR green qPCR is a sensitive and specific assay with a low detection limit for the detection and identification of pathogenic bacteria in food samples (Pakbin et al. 2022). Notably, a novel approach qPCR without pre-enrichment and DNA isolation enables cost-effective, rapid detection of *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*, while significantly reducing processing time and labor requirements compared to the conventional PCR method (Turanoglu et al. 2024). In addition, qPCR can also be combined with functional magnetic beads for foodborne pathogens detection. By performing pre-enrichment with the magnetic beads followed by qPCR analysis, a detection limit of 10 CFU/g was achieved across various food samples, enabling reliable low-level quantification (Dhital and Mustapha 2023).

qPCR does not require post-PCR DNA analysis through gel electrophoresis, thereby reducing the risk of environmental pollution and greatly shortening the time of the whole process. However, qPCR-based detection has inherent limitations, such as the incapability of distinguishing between live and dead cells. Consequently, this method is primarily suitable for bacterial classification, detection, and quantitative analysis in food samples, rather than providing accurate viability assessment (Lombardi et al. 2024). To address the challenge, samples can be pre-enriched in the medium before the qPCR procedure. This step is primarily designed to allow resuscitation or recovery, and subsequent bacterial reproduction for further qPCR detection (Xue et al. 2025), which brings with it the inadequacy of traditional culture-based techniques. Moreover, qPCR technology presents significant practical constraints, including high reagent costs, expensive instrumentation requirements, and demanding technical expertise for proper operation (Nesterova et al. 2024).

Multiplex qPCR

The trend is to develop detection methods for multiple foodborne pathogens, thus making food detection cost-effective and feasible. Multiplex qPCR was derived from the combination of multiplex PCR and qPCR, which can provide simultaneous quantitative analysis of various foodborne pathogens in a single reaction by using more than one pair of primers, reducing the experimental costs and significantly shortening the detection time. Although multiplex qPCR is the preferred characteristic, few effective multiplex qPCR determination methods have been established (Hodzic et al. 2023). According to Sun et al. (2020a, b), rapid detection of diarrheagenic *Escherichia coli* by multiplex qPCR was established, which has the additional advantage of being able to distinguish between viable or dead bacteria, and eliminates the possibility of converting partial results between various samples related to multi-tube determination. TaqMan qPCR methods were developed to simultaneously detect and quantify the most common food-borne pathogens in a single reaction. The results showed that there was no cross-reaction between different pathogens, the specificity was high, and the sensitivity of the method was at least 10 times higher than that of conventional PCR (Hodzic et al. 2023; Xue et al. 2024). It is worth noting that a newly developed TaqMan multiplex qPCR, including a pre-enrichment step, has been proven to be a reliable and efficient method for detecting multiple pathogens (*Staphylococcus aureus*, *Escherichia coli* O157, *Salmonella* spp., and *Listeria monocytogenes*) in food samples, offering significant potential for in vitro diagnostics (Xue et al. 2025). The current trend is to develop detection methods for multiple pathogens, thus making food detection economical, but pursuit of multiplex qPCR has limitations as current commercial thermal cyclers can only distinguish between three or four dyes. As a result, up to four PCR reactions need to be optimized to achieve similar amplification efficiencies to produce reliable multiple reactions.

dPCR

dPCR was first conceived in the 1990s to quantify nucleic acid molecules at the single-molecule level (Sykes et al. 1992). Compared to qPCR, its main advantage is the ability to quantitatively determine target pathogen nucleic acid concentrations without requiring a standard curve. Sample containing nucleotides are partitioned into thousands of individual micro-droplets that contain only one copy of the target DNA molecule of an ideal droplet, or none at all. An independent PCR reaction is then performed on each droplet. After amplification, according to the final fluorescence signal, the number of positive reactions containing the target gene and negative reactions without the target gene is counted by using the thresholds, and the initial concentration of the target sequence is calculated using Poisson statistical analysis (Hindson et al. 2013). According to the limiting dilutions of different samples, PCR method, and Poisson distribution, dPCR is categorized into droplet digital (ddPCR) and chip digital PCR (cdPCR). The ddPCR is performed by using conventional PCR instruments, while cdPCR requires specific instruments for amplification, which to some extent limits its application and promotion. Accordingly, ddPCR has been widely used in pathogen detection, and the reaction principle of ddPCR is illustrated in Fig. 1.

Recent research has also proved the advantages of ddPCR in terms of accuracy and throughput for quantitative detection of foodborne pathogens in food samples. Lei et al. (2020) constructed a ddPCR quantitative method based on four specific genes of *Vibrio parahemolyticus* (*tlh*, *tdh*, *ureR*, *orf8*), and evaluated its detection sensitivity by using artificially contaminated cockle tissues. The detection

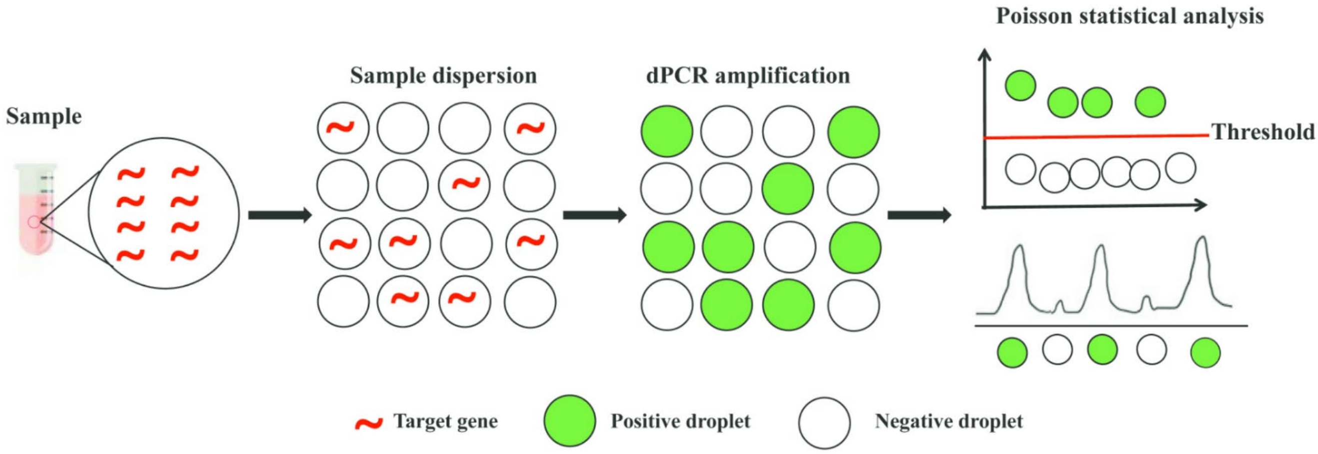

Fig. 1 The principle diagram of ddPCR

sensitivity reached 39 CFU/mL, which is 10 times higher than the qPCR method. The ddPCR is an effective method for detecting and quantifying *Salmonella enterica* subsp., which recently caused a large-scale outbreak in Korea. This method is highly specific and efficient, with a detection limit of 10^1 CFU/mL. Compared to qPCR, the enrichment time for detecting 1 CFU/25 mL was shortened (Kim et al. 2023). Interestingly, a novel 3-plex ddPCR method for simultaneous identification and absolute quantification of *Salmonella* was successfully established. Compared to qPCR, this method significantly reduces the detection limit for artificially contaminated food samples, with a detection limit of 10^1 CFU/mL for lettuce samples, and 10^2 CFU/mL for milk and chicken juice samples, respectively (Fang et al. 2023a, b). Moreover, ddPCR has shown significant ability in detecting low-level targets. Recently, a ddPCR method for quantitative detection of pathogenic populations of *Vibrio parahaemolyticus* was reported, and the detection limit of ddPCR was 1.1 pg/μL, while that of qPCR was 5.6 pg/μL and that of traditional PCR was 8.8 pg/μL (Vidovic et al. 2024). In addition to detecting some pathogenic bacteria, it has also been widely used for medical gene mutation analysis (Bonfiglio et al. 2023), food-derived component analysis (Xu et al. 2022), and transgenic analysis (Głowacka et al. 2016).

Isothermal amplification

Isothermal amplification is a technique of in vitro amplification, in which the target RNA or DNA is amplified specifically to produce a large number of copies at the same constant temperature to reach the detection level, mainly including loop-mediated isothermal amplification (LAMP), recombinase aided amplification (RAA), recombinase polymerase amplification (RPA), nuclear acid sequence-based amplification (NASBA) (As shown in Table 2). In recent years, isothermal amplification has been widely adopted as an effective method for identifying foodborne pathogens (Nesterova et al. 2024). The appeal of this technique is that it does not require specialized and expensive equipment, which is why it is very attractive to many companies engaged in food production.

Table 2 Comparison and applications of different isothermal amplification methods used to the detection of foodborne pathogenic bacteria

Methods	Number of Primers	Enzyme	Pre-Heating	OT (°C)	Reaction Time (min)	Target Template	Detected Pathogens	Limit of detection	Detected foods	Assay time	References
LAMP	4–6	*Bst* DNA polymerase	No	60–65	40–60	DNA	*C. species*; *A. baumannii*; *V. parahaemolyticus*, *S. aureus*, *and Salmonella*	6 CFU per reaction; 10^2 CFU/μL; 5 CFU/25 g	Isolated from hospitalized patient; Contaminated fresh spinach; Shrimp, clam, and crab	NR; NR; 25 min	(Yang et al. 2018; Babu et al. 2020; Du et al. 2022)
RPA	2	Recombinase; polymerase	No	37–42	20–40	DNA	*C. jejuni*; *E. coli* and *S. aureus*	NA; 1 CFU/mL	Artificially contaminated milk and chicken breast Milk	20 min; <5 h	(Geng et al. 2019; Wang et al. 2020)
RAA	1	Recombinase	Yes	30–65	60–90	Circular DNA	*E.coli* O157: H7; *V. Cholerae*;	5.4×10^1 CFU/mL, 46 CFU/mL;	Skim milk; Shrimp	20 min; 50 min	(Fang et al. 2021; Mu et al. 2021)
NASBA	2	RNase H, T7 RNA polymerase	Yes	41	90–120	RNA	*Salmonella*; *Salmonella enterica*	4.89 CFU/25 g; 1.5 CFU/mL	pork and chicken; *Salmonella enterica* cells	NR; NR	(Xue et al. 2022; Zhai et al. 2022)

OT, operating temperature; NR, not reported

Loop-mediated isothermal amplification

LAMP is a nucleic acid amplification technology developed by Notomi et al. (2000) in 2000. Four specific primers were used to identify and bind six specific regions of the target gene, and the chain shift activity of *Bst* DNA polymerase was used to amplify the target DNA at constant temperature (Trinh and Nam 2024).

LAMP is widely used in the detection of *Escherichia coli* O157: H7, *salmonella*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, and other foodborne pathogens (Trinh and Nam 2024). For example, a LAMP method for the specific and rapid detection of *Listeria monocytogenes* in mozzarella cheese was developed, which successfully detected the initial bacterial contamination in food samples at 0.4-4 CFU/g with 100% accuracy after enrichment for 24 h (Busch et al. 2022). *Salmonella* from 69 retail meat and poultry samples of the national antibiotic resistance monitoring system in the United States were rapidly screened by using the constructed LAMP method, and this method was 100% sensitive to cultures (Young et al. 2022). Babu et al. (2020) used the LAMP technology for rapid detection of pathogenic *campylobacter* species. The samples included pure cultures and positive artificially contaminated fresh spinach, and the minimum detection limit was 6 CFU per reaction.

Despite its the advantages of high detection efficiency and low instrument requirements for detecting pathogenic bacteria, LAMP has several limitations (Soroka et al. 2021). Using multiple primers in LAMP detection increases the risk of nonspecific amplification, potentially leading to false-positive results. Additionally, LAMP is unsuitable for short gene sequences, and the design of multiple primers poses challenges for researchers. Another major limitation is the difficulty in achieving multiplexing in a single tube. To overcome these problems, researchers have been working on improving DNA polymerase modification and the primers design to improve the specificity and efficiency of the reaction. Furthermore, the combination of LAMP with advanced molecular technologies (such as RPA, CRISPR/Cas systems) and biosensors has significantly expanded its utility. These improvements have made LAMP a more versatile and efficient method for nucleic acid detection (Yang et al. 2024). For instance, Wang et al. (2024) developed a LAMP-based microfluidic system that can detect dairy ingredients with concentrations as low as 1% within 60 min. This detection method was validated using 35 commercially available samples with 100% specificity and accuracy compared to the qPCR assay.

Recombinase polymerase amplification

RPA technology was developed and is known as a new type of recombinase polymerase amplification detection technology that can replace PCR (Piepenburg et al. 2006). RPA technology is a method to simulate the replication of nucleic acid in T4 phage under constant temperature in vitro. RPA technology initiates DNA replication by utilizing recombinase to facilitate the complementary pairing of single-stranded primers with double-stranded DNA (dsDNA) templates without requiring pre-denaturation of the dsDNA (Tan et al. 2022), and the exposed single-stranded DNA (ssDNA) regions are stabilized by single-stranded binding protein (SSB), enabling polymerase to initiate strand extension. The schematic diagram of the RPA amplification is shown in Fig. 2.

In recent years, RPA technology has been applied to the rapid detection of *salmonella*, *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes*, etc. (Li et al. 2021; Ma et al. 2020). Geng et al. (2019) used real-time RPA with designed special primers to amplify *hipO* gene for rapid and sensitive detection of *Campylobacter jejuni* in food samples, and could detect whether milk was contaminated with *Campylobacter jejuni* within 13 min. However, it often requires a combination of a variety of technologies to enhance detection specificity since RPA is prone to generating false-positive results. For example, a multiple RPA-LFA detection method for *Salmonella*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* in seafood was established, which can simultaneously complete nucleic acid amplification and visual interpretation of the results of three foodborne pathogens within 15 min (Ma et al. 2020). Yin et al. (2020) established a digital RPA method by combining digital PCR and RPA for rapid detection of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enteritidis*, which can determine whether the milk is contaminated by the above three pathogens within 45 min. Compared to other amplification methods requiring complex primer design or template denaturation, RPA exhibits significant advantages in detection and is very suitable for rapid on-site detection, but it also presents certain limitations, such as significantly higher costs, approximately five times that of qPCR (Ndraha et al. 2023). Consequently, the RPA method is mainly used for scientific research and has not yet become an open-access platform. To improve its practical utility and broaden its applicability in the field of food safety, research should focus on developing next-generation RPA technologies with higher specificity in the future, rather than solely depending on companies that provide commercial RPA kits.

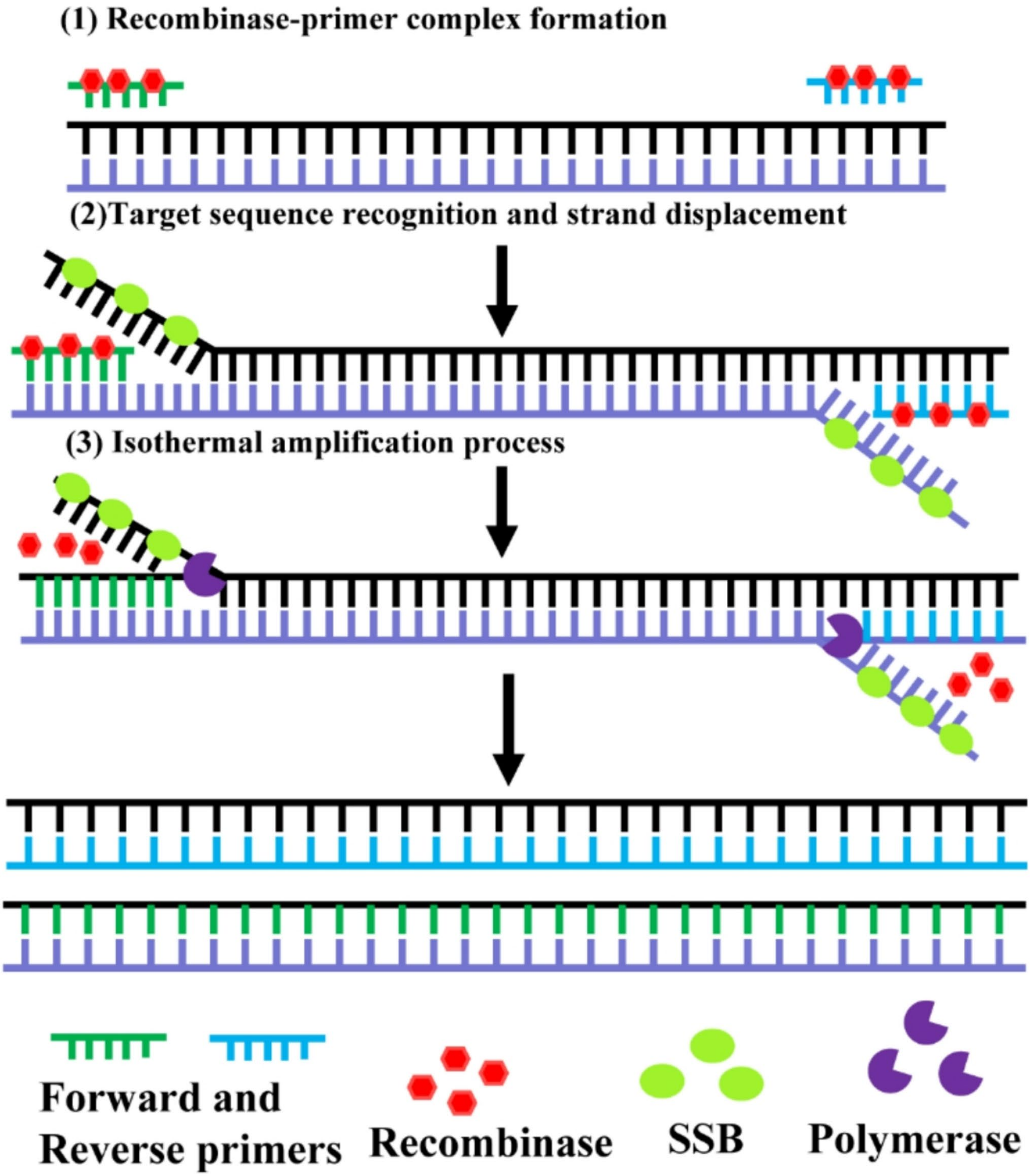

Fig. 2 Schematic diagram of RPA technology

Recombinase aided amplification

The principle of RAA is similar to that of RPA, both of which are techniques for in vitro amplification of nucleic acid target sequences through the interaction of recombinase, recombinase loading factors, and single-stranded binding protein (SSB) with primers and target sequences under isothermal conditions (Zhang et al. 2017). The difference between RPA and RAA is that the recombinase used in RPA is derived from phage T4, while the recombinases used in RAA are derived from bacteria or fungi (Shen et al. 2019).

RAA technology is suitable for the detection of bacterial pathogens (Zhou et al. 2024), due to its advantages, including fast reaction time, cost-effectiveness, high sensitivity, and the ability to efficiently amplify DNA at low temperatures. Mu et al. (2021) established an RAA detection method for *Escherichia coli* O157: H7, with detection limits of 5.4×10^1, 7.9×10^1, and 5.2×10^1 CFU/mL in skim milk, lettuce, and lake water respectively, which has great feasibility in practical detection. Nevertheless, RAA usually requires agarose gel electrophoresis, and even RAA instructions specify that RAA products need to be purified before electrophoresis, which greatly reduces the sensitivity and speed of RAA detection. Previous studies have shown that using RAA in combination with other technologies, such as CRISPR/Cas systems, can optimize detection sensitivity and efficiency (Ndraha et al. 2023). The quantitative analysis of *Salmonella* by the combination of RAA and CRISPR-Cas12a was completed within 75 min, and the detection limit was 6 CFU per mL (Wu et al. 2023).

Nucleic acid sequence-based amplification

NASBA is a constant temperature rapid amplification technology that uses RNA as a template and relies on reverse transcriptase, RNase H, T7 RNA polymerase, and two specific primers for reaction (Kumar 2021). After the first specific primer binds to the target RNA, a complementary DNA single strand is synthesized under the action of reverse transcriptase. Under the action of RNase H, the target RNA is degraded, while the complementary DNA single strand is retained. By combining the single strand of complementary DNA with the second specific primer, a large number of target RNA were synthesized under the action of T7 RNA polymerase (Abdolahzadeh et al. 2019).

In addition to being used for the detection of RNA viruses, NASBA is also widely applied in the detection of pathogenic bacteria due to its strong specificity, high sensitivity, simple operation, and easy contamination (Saravanan et al. 2020). For instance, a NASBA system targeting the specific *xcd* gene of *Salmonella* was designed by using mRNA reverse transcription and successfully detected *Salmonella* in pork, beef, and milk with a minimum detection limit of 10 CFU/25 g (Zhai et al. 2019). Interestingly, its difference from PCR is that it can detect living cells by amplifying messenger RNA, even if genomic DNA is present in the sample. Zhai et al. (2022) established a double-stranded real-time NASBA using a molecular beacon, which could simultaneously detect and identify live cells of *Salmonella* spp. in pork and chicken samples with a detection limit of 4.89 CFU/25 g after pre-enrichment. More importantly, rapid and sensitive detection of viable *Salmonella enterica* by coupling NASBA with a CRISPR/Cas13a system was also developed, which can detect as low as low as 1.5 CFU/mL within 2.5 h in pure cultures (Xue et al. 2022). In this method, CRISPR/Cas13a was employed as the reporter for NASBA. However, there is still a lack of comprehensive data on the application of this new method for detecting foodborne pathogens in food samples, especially when considering the presence of background microbial communities (Ndraha et al. 2023). Therefore, it is imperative to develop technologies that can quickly, accurately, and specifically detect live microbial pathogens in food, and the potential of NASBA in this regard is worth exploring and utilizing (Pang et al. 2024).

DNA hybridization

The principle of DNA hybridization is that the single-strand DNA-labeled probes by the fluorescent dyes form a hetero-DNA molecule with the target DNA or RNA according to the principle of base complementary pairing (Kabiraz et al. 2023). Hybridization of labeled DNA using oligonucleotide probes is cost-effective and requires no specialized instrumentation. Compared to antibodies, oligonucleotide strands as signal probes exhibit stable activity, low cost, simple preparation, and ease of modification on solid-phase carrier surfaces (Xu et al. 2018). Meanwhile, DNA hybridization technology can quickly detect pathogens in food samples without DNA amplification, while avoiding false positive results and aerosol contamination issues. It is a rapid, stable, and sensitive fluorescence-based detection method to detect whether the sample contains the object pathogen by detecting the hybridization signal (See Table 3), even 1×10^{-13} g of homologous DNA can be detected by using this technique (Saravanan et al. 2020). Hu et al. (2022) developed a DNA hybridization-based multiplex and DNA amplification-free sensor using electrical resistance microsphere counter as the ultrasensitive signal readout for the simultaneous detection of *L. monocytogenes*, *Salmonella*, and *S. aureus* in egg samples. The detection limit of this method is 20 CFU/mL for *L. monocytogenes*, 89 CFU/mL for *Salmonella*, and 94 CFU/mL for *S. aureus*, respectively. Moreover, a new peptide nucleic acid (PNA) fluorescence in situ hybridization method was developed for the specific detection of *Salmonella* species. The method presented a specificity of 100% and a detection limit of 0.5 CFU/25 g of food sample (Sousa et al. 2024).

Genomic sequencing

Genomic sequencing technology has experienced the development of three generations of technologies since 1977,

Table 3 Application of DNA hybridization-based, genomic sequencing-based, and CRISPR/Cas-based detection for foodborne pathogens

Methods	Detected Pathogens	Limit of detection	Detected food	References
DNA hybridization	*L. monocytogenes*, *Salmonella*, *S. aureus*; *Salmonella*	20 CFU/mL, 89 CFU/mL, 94 CFU/mL; 0.5 CFU/25 g	Eggs; Contaminated fresh ground beef	(Hu et al. 2022; Sousa et al. 2024);
Genomic sequencing	*L. monocytogenes*; *L. monocytogenes* and *S. Enteritidis*	NR; NR	Refrigerated ready-to-eat foods; Isolated from hospitalized patient	(Parra-Flores et al. 2025; Hong et al. 2024)
CRISPR/Cas	*V. Parahaemolyticus* *S. aureus*	6 CFU/mL; 16 CFU/mL	Orange juice and frozen shrimp; Eggs, milk, and pork	(Wang et al. 2025; Wei et al. 2023);

NR, not reported

such as Sanger sequencing, next-generation sequencing (NGS), and nanopore sequencing (NS), and has made great progress (Zhou et al. 2022). Among them, NGS is mainly used to determine the whole genome sequence of isolated microorganisms (whole genome sequencing), to determine the sequence of many microorganisms by metagenomics in the sample, or to determine a specific microbiome by 16 S rRNA, ITS, or any other biomarker (bacteria, fungi, or viruses) (Grinevich et al. 2024; Park et al. 2023). The third-generation NS, primarily offered by Oxford Nanopore Technologies (ONT; Oxford, United Kingdom) as a commercial platform, has been widely used in the detection of foodborne pathogens in recent years (Hurtado et al. 2025).

Genomic sequencing has been widely used for analyzing the characteristics of foodborne pathogenic bacteria (See Table 3), including *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., and *Vibrio parahaemolyticus*, providing profound insights into the genetic composition of these microorganisms and also revealing the emergence mechanism and spread a path of drug-resistant bacterial pathogens (Ahmed et al. 2025; Hurtado et al. 2025; Parra-Flores et al. 2025). For instance, Buytaers et al. (2024) developed a metagenomics approach based on whole-genome amplification and adaptive sampling, which can characterize foodborne pathogens at the strain level without culture enrichment or isolation, thereby enabling timely intervention in case of an outbreak. The results also demonstrated that nanopore sequencing with adaptive sampling outperforms NGS shotgun metagenomics. Another study also revealed that NS exhibited comparable accuracy to Illumina sequences in whole genomic sequencing-based genotyping of *L. monocytogenes* and *S. Enteritidis* isolates, effectively discriminating among bacterial strains from outbreaks (Hong et al. 2024). Notably, an approach for ultra-rapid nanopore whole genomic sequencing combined with an optimized sample preparation protocol, can sequence a genome and detect pathogen variants within 8 h, which is 46–50% higher than previous records (Goenka et al. 2022). Therefore, genomic sequencing technology plays an increasingly important role in determining the source of contamination events, tracing the origin of food safety incidents, detecting and identifying food safety emergencies, analyzing virulence and pathogenic characteristics (Mather et al. 2024), as well as genes of interest-including those linked to antibiotic resistance (Aworh et al. 2025).

CRISPR/Cas-based detection technique

CRISPR/Cas is an acquired immune system presented in most bacteria and archaea (Barrangou et al. 2007). CRISPR not only has outstanding genome editing capabilities, but also shows great potential in the field of foodborne pathogen detection detection because of its advantages of good specificity, high sensitivity, and convenient detection (Mao et al. 2023) (See Table 3). As a new nucleic acid detection technology, CRISPR technology can be combined with other methods such as fluorescence biosensors, colloidal gold strips, colorimetric biosensors, and nanomaterials to develop various detection platforms (Lu et al. 2024; Saleh et al. 2024).

According to the composition and function of the Cas effector, reported CRISPR/Cas systems include Cas9, Cas12, Cas13, and Cas14 as the effectors, respectively (Harrington et al. 2018; Yin et al. 2023). Cas9, which can specifically recognize and cut target dsDNA, was developed as a gene-editing tool. There have been numerous reports of researchers using Cas9 to detect foodborne disease-causing microbes (Fan et al. 2021; Rathore et al. 2024). For instance, a CRISPR/Cas9-triggered isothermal amplification method for fluorescence detection of *Escherichia coli* O157:H7 was developed with a sensitivity of 40 CFU/mL (Sun et al. 2020a, b). Compared with real-time PCR, this method showed a wider detection range and lower limit of detection.

Owing to its trans-cleavage activity and RNA-mediated non-specific cutting activity on ssDNA or dsDNA of Cas12, the CRISPR/Cas12a system has become one of the most widely used CRISPR analysis tools and has shown significant potential in developing new methods to detect foodborne pathogens (Shin et al. 2022). Cas12-based fluorescent biosensors, such as trans-cleavage of fluorescent probes, were developed for rapid detection of pathogenic

Escherichia coli and *Listeria monocytogenes* serotype 4c (Li et al. 2022; Rathore et al. 2024), as illustrated in Fig. 3. This approach utilizes a single-stranded DNA (ssDNA) fluorescent reporter coupled with a quencher to detect the test samples containing target genes from pathogenic microorganisms. In the presence of the target gene in the sample, the Cas12a and crRNA complex are activated and initiate cleavage of the complementary gene sequence. Upon activation, the Cas complex proceeds to cleave any single-stranded DNA, including the ssDNA reporter. Once the fluorescent reporter is separated from the quencher, it becomes excited under ultraviolet light and emits a fluorescent signal. The presence of fluorescence in the sample indicates the existence of the target gene in the sample. In addition, a rapid detection method for pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* was developed by combining RAA technology with CRISPR/Cas12a and using a handheld fluorescence analyzer, which can complete the detection of food-borne pathogenic bacteria with high sensitivity and specificity within 40 min, and the detection limit is 10 copies/reaction (Liu et al. 2021). Wei et al. (2023) established a CRISPR/Cas12-based magnetic relaxation switch biosensor for methicillin-resistant *Staphylococcus aureus* detection in food. The detection limit of this biosensor in food samples is as low as 16 CFU/mL without the need for nucleic acid preamplification. It has high sensitivity and accuracy, which is important for reducing false positives in *Staphylococcus aureus* detection.

Similar to Cas12, Cas13 possesses reverse-cutting activity for ssRNA instead of DNA. Based on CRISPR/Cas13a system, methods have been developed for the detection of foodborne pathogenic bacteria. Zhou et al. (2020) developed a CRISPR/Cas13a detection method by combining PCR technology and fluorescent detection, which is used for the detection of *Staphylococcus aureus* in food. The detection time is less than 4 h, and the detection limit is 1 CFU/mL. A PCF (PCR-CRISPR-Fluorescence based nucleic acid) detection method was proposed, which showed excellent sensitivity with no cross-reaction with other common foodborne bacteria, and can detect *Salmonella* genomic DNA with a minimum of 10^1 aM or 10^0 CFU/mL *Salmonella* spp. in 2 h (Gao et al. 2021).

Cas14a, notable for its small size, exhibits characteristics similar to those of Cas12a and strong single-base specificity, but is limited by the fact that it only recognizes single-stranded DNA (Chen et al. 2024). Meng et al. (2024) reported a Cas14a-based isothermal amplification strategy, named integrating competitive annealing mediated isothermal amplification-mediated CRISPR-Cas14a isothermal detection platform, which can release a large number of visible detection signals for rapid, accurate detection of *S. pyogenes* with high sensitivity and specificity of 10^2 aM, suggesting that it is a promising and practical pathogen diagnosis technique. Additionally, Wang et al. (2025) developed a fluorescent biosensor based on the side chain Cas14a cleavage activity and Exo III cleavage reaction with a wide linear range for detecting pathogenic *Vibrio parahaemolyticus*, with a detection limit of 6 CFU/mL, which also has strong advantages in detecting *Vibrio parahaemolyticus* in complex substrates.

As mentioned above, CRISPR/Cas enzymes typically have sequence specificity and can be combined with various nucleic acid amplification techniques or biosensors, which not only improve the sensitivity and specificity of detection methods, but also expands its range of applications, making it possible for laboratory to field application to be achieved (Deng et al. 2024; Shin et al. 2022). However, off-target activity remains a major challenge in CRISPR/Cas-based developing detection methods, the detection time and sensitivity are still constrained by the target amplification requirements, and it is necessary to improve the detection efficiency by integrating the amplification steps (Chen et al. 2024).

Other molecular diagnostic techniques

Apart from all these, there are some alternative techniques such as increasingly mature high-throughput omics techniques, including transcriptomics, secretomics, proteomics, and metabolomics, which have been widely applied in the rapid identification and study of stress resistance

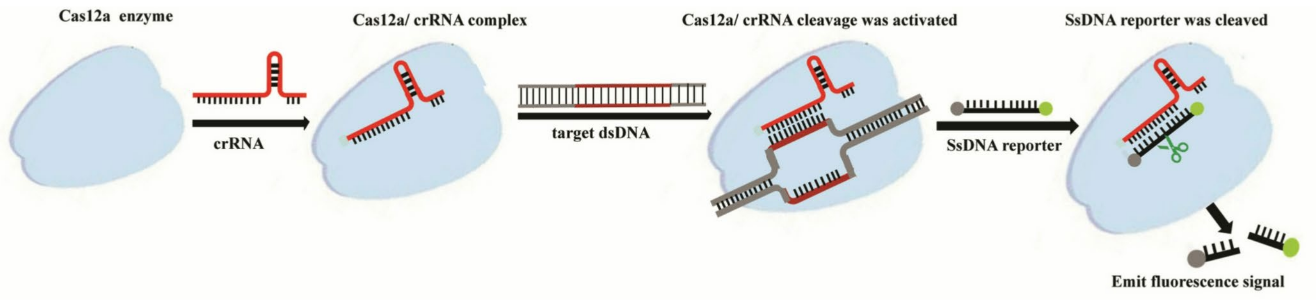

Fig. 3 Schematic diagram of CRISPR/Cas12a combined with fluorescent probes

mechanisms of foodborne pathogens such as *Salmonella* spp. *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Fang et al. 2023a, b; Zhao et al. 2024). In recent years, photoelectric bio-sensing of nucleic acid technology has been developed and applied to the detection of pathogenic bacteria in food (Dong et al. 2024). Sheng et al. (2019) developed a transcriptional biosensor with light-up RNA aptamers, which can perform sensitive, rapid quantification, and culture-free detection of food pathogens without isolation and purification.

In addition, more rapid and sensitive methods for the detection of viable pathogens in food are continually being sought. A new detection technology, phage amplification, can distinguish live cells from dead cells effectively by using the high specific recognition ability of phage to host bacteria, and improve the accuracy of detection results (Bumunang et al. 2023). In particular, bacteriophage amplification has shown unique advantages in the detection of pathogenic bacteria in the live uncultivated state. Combining phage amplification and lysis with PCR/qPCR, immunoassays, or enzyme protein analysis detection methods appears to be the most promising rapid approach. If the host cell is undergoing metabolism, phage amplification will occur and the pathogen cell will eventually lyse, releasing detectable intracellular components such as ATP, enzymes, host DNA, or progeny phages (Foddai and Grant 2020). The results showed that the minimum detection limit of viable *Salmonella* enteritidis by the qPCR method was 8 CFU/25 g (Garrido-Maestu et al. 2019). For example, Kim et al. (2024) developed an effective detection method for three pathogenic bacteria *Salmonella Typhimurium Escherichia coli* O157:H7, and *Bacillus cereus* in contaminated kimchi cabbage that leverages bacteriophage-assisted live-cell lysis and multiple PCR amplification of exposed genomic DNA, and it was demonstrated to detect foodborne pathogens such as *E. coli* O157: H7, *S.* typhimurium and *B. cereus* within 3 h of specific food samples at a concentration of 10^3 CFU/mL.

Conclusions and perspectives

Foodborne pathogens are one of the main factors leading to food safety problems. Effective, rapid, and accurate detection methods of foodborne pathogens are crucial, which provide strong support for timely response to food-borne disease outbreaks, effective prevention, and control of foodborne diseases. As shown in Table 4, the advantages and limitations of various rapid molecular detection methods for foodborne pathogens are summarized. Molecular detection techniques based on DNA or RNA have been developed and widely applied due to its advantages of strong specificity, high accuracy, and fast detection speed, such as PCR and its derivative technologies, DNA hybridization as well as DNA microarray, but such methods are limited by professional operators and specialized instruments. With the development of isothermal amplification technology, nucleic acid detection technology is being optimized to the direction of simple, portable, fast, and sensitive instant detection without large instruments and equipment and professional operators. Especially after the birth of CRISPR/Cas detection technology, a variety of biological analysis detection platforms have been developed according to nucleic acid detection, which has good development prospects in the field of foodborne pathogen detection.

In addition, the rapid development of genomic sequencing technology provides a more powerful tool for the detection of foodborne pathogens, which can determine the sequences of multiple DNA molecules at once, achieving high-throughput and high-precision analysis of microbial genomes (shown in Table 4). The technology not only improves the speed of detection, but also enables the discovery of new and previously unidentified pathogens, providing important support for timely detection, traceability research, and risk assessment.

In conclusion, although molecular biotechnology still has shortcomings in the detection of food pathogens, it is still playing an increasingly important role in the detection of foodborne pathogens. With the continuous progress of technology and the deepening of application, it will provide a more reliable and efficient means for food safety. Therefore, the future development and application of new technologies should continue to be strengthened in the following aspects, and the sensitivity and specificity of detection technologies should be improved to better address the challenges of foodborne infections.

(i) Combined with other new technologies, such as immunology technology, optical technology, electrochemical technology, and biosensor technology, is the development trend of food-borne pathogens detection technology in the future to develop cheaper, faster, more accurate, and more portable detection technology through the complementarity of different detection methods. For example, based on the CRISPR technology, nucleic acid isothermal amplification technologies such as LAMP and RPA are used to amplify target genes and achieve further amplification of signals. CRISPR technology will develop in the direction of multiple detection and integrated detection, to achieve a more stable reaction system and simpler practical operation, which will help develop it into a commercial kit. In addition, the combination with proteomics, metabolomics, and other technologies will also provide more new markers and detection methods for pathogenic bacteria detection.

Table 4 Summary of advantages and limitations of various rapid molecular detection methods for foodborne pathogens

Methods	Advantages	Limitations
PCR	Primer design is simple, highly specific, and widely applicable.	High temperature requirements, susceptibility to pollution, and long time consumption.
Multiplex PCR	Simultaneously detect multiple foodborne pathogens with strong specificity, saving time and low cost.	High requirements for primers design and the amplification effect is low, severe false positives, and inability to quantify.
qPCR	High specificity and automation, wide detection range, fast detection speed and real-time observation of detection results.	Numerous interference falsepositives, the expensive instrument equipment and high technical requirements for operators.
Multiplex qPCR	Quantitative detection of a variety of foodborne pathogens by a single qPCR reaction.	Complex primer design, high usage cost, the expensive instrument equipment and high technical requirements for operators.
dPCR	High specificity, reproducibility, absolute quantitative analysis, low sample requirement	High cost, limited throughput, complex operation.
LAMP	High amplification efficiency, strong specificity, visual detection, low equipment requirements, low cost, high low tolerance.	Complex primer design, low throughput, non-specific amplification, easy to produce aerosol pollution, easy to produce false positive.
RAA	High specificity, high sensitivity, constant temperature detection.	Expensive reagents, longer primers, not suitable for short sequence nucleic acids detection.
RPA	Simple equipment, high specificity, short assay time, high sample tolerance.	Expensive reagents, longer primers, non-specific amplification, poor quantitative separation rate.
NASBA	High sensitivity, strong specificity, fast detection speed, not easy to be contaminated.	Need reverse transcriptase, higher cost, complex reaction components, and ineffective for detecting pathogens.
DNA hybridization	High precision, greatly reduce the detection time, high throughput.	Complicated operation process, high the design cost of probes, the stability is not ideal when the sample matrix is complex, low sensitivity.
Genomic sequencing	Reliable and accurate results, suitable for testing/identification, suitable all microbial communities (rare or uncultivated group), generating large amounts of useful genetic data	Equipment and operation are relatively expensive, high requirements for sampling instruments and personnel.
CRISPR/Cas	High precision, high specificity, even down to the single-nucleotide accuracy.	High cost of equipment and reagents, complex primer design, high requirements for personnel, difficulty in implementing multiple detection.

(ii) Future molecular diagnostic techniques will aim to further improve detection sensitivity and accuracy. This includes optimizing existing detection technologies, such as PCR, LAMP, and improving detection efficiency by improving primer design and optimizing reaction conditions for avoiding cross-reaction, mutual interference of different primers, and false positives. At the same time, new detection targets and markers will also be explored to achieve more accurate detection of pathogens.

(iii) Automation and intelligence are important trends in the development of molecular biology technology in the future. With the continuous development of robotics, artificial intelligence, and testing instruments, pathogen detection will be promoted in the direction of automation and intelligence. Through the integrated detection equipment and intelligent analysis system, the whole process from sample processing to result output can be automated, which greatly improves the detection efficiency and accuracy.

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Declarations

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