

Article

Polystyrene Microsphere-Labeled Lateral Flow Assay for the Visual Detection of Foodborne Pathogens

Lingmei Zhang ^{1,2}, Wanwei Qiu ^{1,*}, Lu Lu ¹, Jinghui Liu ¹, Zhipeng Zou ¹, Litao Yang ^{3,*}  and Haobo Sun ^{4,5,*}

¹ School of Biomedicine and Health, Anhui Science and Technology University, Chuzhou 233100, China; zhanglm05@126.com (L.Z.); lulu09052026@126.com (L.L.); liujh4010106@163.com (J.L.); zouzhip@outlook.com (Z.Z.)

² School of Food Science and Engineering, Anhui Science and Technology University, Chuzhou 233100, China

³ National Center for the Molecular Characterization of Genetically Modified Organisms, Joint International Research Laboratory of Metabolic and Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

⁴ Shanghai Key Laboratory for Nucleic Acid Chemistry and Nanomedicine, Institute of Molecular Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China

⁵ School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

* Correspondence: qiuww@ahstu.edu.cn (W.Q.); yylltt@sjtu.edu.cn (L.Y.); haobosun@sjtu.edu.cn (H.S.)

Abstract

With the increasing emphasis on food safety and health, it has become particularly important to develop rapid, sensitive and low-cost detection methods for foodborne pathogens. Lateral flow assay (LFA) has shown great potential in the field of point-of-care testing (POCT) due to its rapidity, portability and low cost. However, traditional gold nanoparticles (AuNPs)-labeled LFAs face challenges such as insufficient signal strength when detecting nucleic acids. In this study, LFA labeled with polystyrene microspheres was constructed targeting the specific nuc gene of *Staphylococcus aureus* for the detection of double-stranded PCR products. Unlike traditional AuNPs that pair antibodies through physical adsorption, polystyrene microspheres adopt a covalent coupling strategy, significantly enhancing probe stability and signal strength. Under the optimized conditions, the detection limit was calculated to be 7.28×10^2 CFU/mL, which was approximately 10 times higher than that of the AuNP-based strip. This method demonstrated excellent specificity, reproducibility (RSD < 5%) and stability. In the practical application of artificially contaminated milk samples, the detection performance of polystyrene microsphere-based strips was better than that of AuNP-based strips. This study provides an efficient and easy-to-operate solution for the visual detection of foodborne pathogens.

Keywords: lateral flow assay; polystyrene microspheres; PCR products; food safety



Received: 8 January 2026

Revised: 30 January 2026

Accepted: 6 February 2026

Published: 10 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

As global food production, processing, and consumption continue to expand, foodborne diseases have emerged as a major public health concern worldwide, foodborne illnesses and fatalities caused by pathogenic bacteria remains high every year [1,2]. *Staphylococcus aureus*, a significant foodborne pathogen, produces enterotoxins that can cause severe gastrointestinal disturbances, including nausea, vomiting, diarrhea, and even death [3]. Contamination of food by *Staphylococcus aureus* is commonly associated with dairy products, meats, and eggs, posing a serious threat to food safety [4]. Although traditional culturing methods are widely employed, they are often time-consuming, labor-intensive, and impractical for rapid on-site detection [5,6]. Therefore, the development of rapid,

sensitive, and user-friendly pathogen detection methods is of utmost importance. Current detection techniques for foodborne pathogens predominantly include immunological assays, molecular biology methods and biosensors [7–9]. Immunological methods such as enzyme-linked immunosorbent assay (ELISA) and traditional antigen/toxin detection techniques are widely used but are hindered by their complexity, long processing times, and low sensitivity [10]. Molecular methods such as real-time/quantitative PCR and digital PCR offer high sensitivity and specificity but typically require specialized and expensive laboratory equipment [11–13]. Although portable conventional PCR and isothermal amplification can also achieve the amplification of target sequences, they often require subsequent detection steps such as electrophoresis [14,15].

As a convenient, cost-effective, and user-friendly detection platform, lateral flow biosensor has gained increasing attention [16–18]. LFA combines paper-based capillary action, immune (or nucleic acid) recognition, and visual signal output, allowing results to be read by the naked eye without the need for complex instrumentation [19,20]. This makes it highly suitable for on-site testing and food safety monitoring in resource-limited environments [21–24]. One of the core components of LFA is the “label” or “reporter” system, which determines the visualization, sensitivity, and stability of the detection signal [25]. Traditional LFAs often use AuNPs as the labeling material. AuNPs are favored for their distinct color, excellent biocompatibility, and independence from complex equipment, making them widely used for the rapid detection of pathogens and toxins [26–28].

However, AuNP-based labeling also has certain limitations [29]. For instance, the attachment of antibodies primarily relies on physical adsorption, which may be influenced by environmental conditions (such as pH, salt concentration, etc.), potentially affecting the stability and consistency of the complex [30]. Moreover, when the target for detection is PCR amplification products (nucleic acids) rather than proteins/antigens, the requirements for labeling stability, binding efficiency, and signal strength become even more stringent. Some studies have also pointed out that the detection limits and sensitivity of traditional LFA may still fall short in certain applications [31,32]. To address these issues, recent research has focused on improving LFA design or switching labeling materials to enhance performance [33–35]. In recent years, researchers have increasingly turned their attention to alternative types of nanoparticles/microparticles for labeling, such as fluorescent nanoparticles, up-conversion nanoparticles (UPNs), quantum dots (QDs), magnetic nanoparticles, and colored polystyrene microspheres [20,36–39].

Among these alternatives, polystyrene microspheres have emerged as highly promising LFA labeling materials due to their easy synthesis, flexible surface functionalization, diverse color options, low cost, and ease of storage [40–42]. By covalently coupling antibodies onto the surface of polystyrene microspheres through chemical methods such as carboxyl-EDC/NHS activation, more stable, uniform, and interference-resistant complexes can be achieved compared to simple physical adsorption, thereby improving signal stability and assay sensitivity [43–45]. In recent years, dyed latex microspheres have been widely adopted as colorimetric labels in LFAs for diverse targets, including viral biomarkers, toxins, and metal ions, benefiting from their high dye-loading capacity and visual contrast on nitrocellulose membranes [40,42,43]. In addition to colorimetric readout, polystyrene microspheres can also serve as carriers for fluorescent or lanthanide chelates, enabling further signal amplification and quantitative detection when combined with portable readers, which supports the development of more sensitive point-of-care platforms [46]. Lateral flow assays have also been developed specifically for *Staphylococcus aureus* detection using various recognition and reporting strategies. These include nucleic acid-based LFA platforms for rapid detection of *Staphylococcus aureus* using fluorescence probes [47] and immunochromatographic LFAs targeting *Staphylococcus aureus* surface antigens [48],

highlighting the feasibility of LFA-based detection for this important foodborne pathogen. In this work, we further extend the application of polystyrene microsphere labeling to a PCR-LFA platform for enhanced visual detection of *Staphylococcus aureus* PCR products.

In this study, lateral flow strips labeled with polystyrene microspheres were designed and constructed, while traditional AuNP-labeled LFA as control. The performance of the two labeling systems was compared under the same PCR target, identical chromatographic structure, and the same detection conditions, evaluating parameters such as detection limit, specificity, reproducibility, stability, and applicability to food samples. The results indicate that the polystyrene microsphere-labeled LFA not only offers superior sensitivity compared to the AuNP-based strips but also shows enhanced advantages in terms of practicality, providing a low-cost, highly sensitive, and field-operable solution for food safety testing.

2. Experimental Section

2.1. Optimization of PCR Amplification System

The PCR reaction mixture had a total volume of 50 μL , containing 25 μL of $2\times$ PCR Master Mix. This mix included 0.1 U/ μL Taq DNA polymerase, 0.2 mM dNTPs, MgCl_2 , and a buffer solution, providing the necessary components for the PCR reaction. The concentrations of the forward and reverse primers were optimized in subsequent experiments. This reaction system served as the foundation for standard PCR protocols, and the component concentrations were further adjusted as needed to achieve optimal amplification results. The amplification process employed $1\times$ TAE buffer and 4S Green Plus DNA dye for electrophoretic analysis on a 3% agarose gel, with a negative control (using water in place of the DNA template) to validate the amplification.

In order to optimize the PCR amplification efficiency, several critical reaction parameters were tested using gradient settings. Magnesium ion concentrations were varied at 2.5, 3, 3.5, and 4 mmol/L to enhance both sensitivity and specificity of the amplification reaction. Primer concentrations were set at 0.5, 1, 1.5, and 2 μM to optimize reaction specificity and minimize the formation of primer dimers. A gradient of annealing temperatures ranging from 50 $^{\circ}\text{C}$ to 60 $^{\circ}\text{C}$, with nine different temperature settings, was employed to ensure optimal primer-template binding. All optimized conditions were validated through electrophoresis, assessing the intensity and specificity of the amplified bands to ensure the final PCR conditions were optimal.

2.2. Conjugate Preparation and Labeling

2.2.1. Conjugation of AuNPs with FITC Antibody

AuNPs were synthesized using the classical trisodium citrate reduction (Turkevich–Frens) method to obtain stable and monodisperse nanoparticles (~20 nm) and were used as a conventional reference label in this study [49]. After preparation, the AuNPs were conjugated with a specific antibody. The procedure began by preparing a pH 8.0 coupling buffer by adjusting ultrapure water with 1.0 M K_2CO_3 . The 20 nm AuNP colloid was concentrated by centrifugation at $12,000\times g$ for 10 min (5-fold concentration), the supernatant was discarded, and the pellet was resuspended in 200 μL of the pH 8.0 H_2O . Then, 10 μL of rabbit anti-FITC antibody (1 mg/mL) was added to the solution, and the mixture was incubated on a shaker at room temperature for 1 h. Following this, 2 μL of 10% BSA solution was added to block non-specific binding, and the reaction was allowed to proceed for 30 min. The mixture was then centrifuged at 12,000 rpm for 12 min at 4 $^{\circ}\text{C}$, and the supernatant was discarded. The resulting precipitate was washed with 200 μL PBS buffer containing 1% BSA, and this washing step was repeated twice. Finally, the precipitate was resuspended in 200 μL Eluent Buffer containing 0.076 g $\text{Na}_3\text{PO}_4\cdot 12\text{H}_2\text{O}$, 0.5 g BSA, 1 g sucrose, 22.7 μL Tween-20, and 10 mL dd H_2O , and stored at 4 $^{\circ}\text{C}$ for later use.

2.2.2. Conjugation of Polystyrene Microspheres with FITC Antibody

In this study, carboxylated blue polystyrene latex microspheres (nominal diameter: 100 nm; stock concentration: 10 mg/mL) were activated through chemical methods and conjugated with a specific antibody. First, the polystyrene microspheres were pre-treated by centrifuging a 1 mL microsphere suspension (2 mg/mL) at 10,000 rpm for 8 min, discarding the supernatant, and washing the pellet with 1 mL of 0.01 mol/L PBS buffer at 8000 rpm.

This washing step was repeated twice. Next, a solution containing 9.6 mg EDC, 5.43 mg NHS, and 1 mL MES was prepared, and the microsphere pellet was fully dissolved in the solution. The mixture was incubated on a shaker at room temperature for 15 min, followed by centrifugation at 8000 rpm for 6 min. The supernatant was discarded, and the pellet was washed again with 1 mL of 0.01 M PBS buffer. Subsequently, 10 µL of 1 mg/mL rabbit anti-FITC antibody was added to the microsphere suspension, which was then thoroughly mixed and sonicated. The reaction continued on a shaker for 3 h, after which the microspheres were centrifuged, and the unbound antibody was removed by washing twice with 1 mL of 0.01 mol/L PBS. The final pellet was resuspended in 1 mL Eluent Buffer and stored at 4 °C for later use. This process enables the stable conjugation of the FITC antibody to the polystyrene microspheres, which is essential for the subsequent detection of PCR products using lateral flow assays.

2.3. Construction and Optimization of Lateral Flow Assay

2.3.1. Construction and Optimization of AuNP-Based LFA

The core components of the LFA strips include the sample pad, conjugate pad, NC membrane, and absorbent pad. Each part was optimized in terms of material and size to meet the specific detection requirements. Detection lines (T line) and control lines (C line) were formed on the NC membrane by a dispenser (setup 0.5 µL/cm). The C line was coupled with goat anti-rabbit IgG (1 mg/mL), and the T line was coupled with streptavidin (SA, 1 mg/mL). After coating, the strips were left to dry at room temperature overnight. To prepare the sample pads, a buffer solution consisting of 1.168 mL Triton X-100, 3.94 g Trizma, 37.5 µL 2 M NaCl, and 500 mL ddH₂O was used to soak the sample pads for at least 1 h. The pads were then dried in an oven at 37 °C and stored at 4 °C. Afterward, the NC membrane, conjugate pad, sample pad, absorbent pad, and PVC backing were assembled and cut into strips approximately 4 mm wide. The strips were sealed in plastic bags and stored at 4 °C for further use.

To enhance the detection performance of the PCR-LFA strips, key construction parameters were systematically optimized. First, the final concentration of FITC antibody coupled with AuNPs was optimized, and the DNA was extracted and amplified by PCR. The strips were used to detect the PCR products, and the band intensity was compared under different coupling conditions. Next, the concentration of streptavidin (SA) on the T line was optimized to determine the optimal coating condition for improved detection performance. Finally, the buffer concentration was optimized by testing different PBS concentrations for sample application.

2.3.2. Construction and Optimization of Polystyrene Microsphere-Based LFA

The construction of PCR product detection strips based on polystyrene microspheres follows the same procedure as that of colloidal gold-based strips. To improve the performance of PCR-LFA strips, we systematically optimized key parameters, including the concentration of polystyrene microspheres, FITC antibody coupling, T line streptavidin (SA) concentration, and buffer composition. The microsphere concentration was optimized by testing different amounts of microspheres during activation and conjugation, followed by DNA extraction and PCR amplification. The FITC antibody coupling con-

dition was optimized by evaluating different final antibody concentrations for coupling with the polystyrene microspheres. In addition, the SA concentration on the T line and the running buffer formulation were further optimized to improve signal intensity and reduce background interference. These optimization steps ensured that the strips provided highly specific and sensitive detection signals, thereby improving the detection efficiency of PCR products.

2.4. Analytical Performance

To assess the performance of the two PCR product detection strips, both the colloidal gold-based and polystyrene microsphere-based strips were tested for specificity, sensitivity, reproducibility, and stability based on the optimized construction conditions.

For the specificity testing, the ability of the strips to recognize target DNA was evaluated using non-target bacterial strains, including *Shigella flexneri*, *Shigella sonnei*, *Escherichia coli*, *Salmonella*, and *Pseudomonas putida*, as negative controls. DNA was extracted from *Staphylococcus aureus* and other control strains and amplified under optimal PCR conditions. Conjugates were applied to the conjugate pad, and 100 μ L of sample buffer containing PCR products was added to the sample pad of the test strip. After incubation for 10 min, the sample pad was washed with buffer. The appearance of visible blue (or red) bands at both the test line (T line) and control line (C line) was observed visually. The intensity and appearance of the bands were compared to assess the specificity of primer binding to the target DNA. Quantitative analysis of the T line signal intensity was performed using a strip reader to provide a more precise measurement.

The sensitivity of the PCR-LFA method was evaluated by adjusting the concentration of PCR products to determine the lowest detectable concentration. A series of dilutions of *Staphylococcus aureus* bacterial suspension, starting from 5.6×10^6 CFU/mL, were prepared by serially diluting to concentrations ranging from 5.6×10^6 to 5.6×10^1 CFU/mL, with a blank sample included. DNA was extracted from the different bacterial suspensions, followed by PCR amplification under optimized conditions and detection using the lateral flow assay strips. The color intensity at both the T line and C line was visually examined, and the results were used to assess the sensitivity of the detection method. This approach ensured the high efficiency and reliability of the PCR product detection system.

Reproducibility was assessed by testing *Staphylococcus aureus* DNA at concentrations ranging from 5.6×10^6 to 5.6×10^3 CFU/mL. Each concentration was tested in six parallel experiments. The peak area signal values were recorded using a strip reader.

The stability of the lateral flow assay strips was evaluated by testing *Staphylococcus aureus* DNA at concentrations of 5.6×10^6 and 5.6×10^4 CFU/mL, along with a blank control, over a six-month period. Tests were performed every two months, and color intensity changes were compared alongside signal values measured by a strip reader.

All experiments were conducted using consistent operational procedures, buffer systems, and result interpretation criteria to minimize the risk of false positives or false negatives due to procedural differences. To ensure data accuracy and reproducibility, each concentration was tested in triplicate using strips from the same batch. The resulting data were then used to establish the detection limit (LOD), linear range, and specificity of the method.

2.5. Practical Sample Detection

To evaluate the applicability and detection performance of the constructed PCR product detection strip in real samples, spiking recovery experiments were conducted using sterile milk as a model matrix for detecting *Staphylococcus aureus*. Commercially purchased sterile milk was divided into several 900 μ L aliquots. A 10-fold dilution of *Staphylococcus au-*

reus culture was prepared, and 100 μL of the diluted bacterial solution was added to the sterile milk, resulting in a final bacterial concentration range of 5.6×10^5 to 5.6×10^1 CFU/mL. The samples were then incubated at 37 °C with shaking for 2 h in an aerobic environment, followed by DNA extraction. The extracted DNA was subjected to PCR amplification, and the PCR products were analyzed using the lateral flow strip. During the detection process, 100 μL of the spiked sample was added to the sample pad of the test strip, and the strip was incubated at room temperature for 15 min. The color changes in the T line and C line were then observed for both qualitative and quantitative analysis. For qualitative analysis, the T line was visually inspected for color development, while for quantitative analysis, the grayscale values of the T/C lines were measured using the strip reader.

3. Results and Discussion

3.1. Principle of PCR Product Detection Using Polystyrene Microspheres

The principle of the polystyrene microsphere-labeled PCR-LFA detection platform constructed in this study is shown in Figure 1. PCR amplification was performed using a dual labeled primer system targeting *Staphylococcus aureus*-specific nuc genes. The forward primer, labeled with FITC at the 5' end (5'-FITC-GCATGTAGTGTGATGCAGGTT-3'), and the reverse primer, labeled with biotin at the 5' end (5'-Biotin-AGCCAAGCCTTGACGAAGACT-AAAGC-3'), resulted in a double-stranded DNA (279 bp) with FITC and biotin tags at both ends.

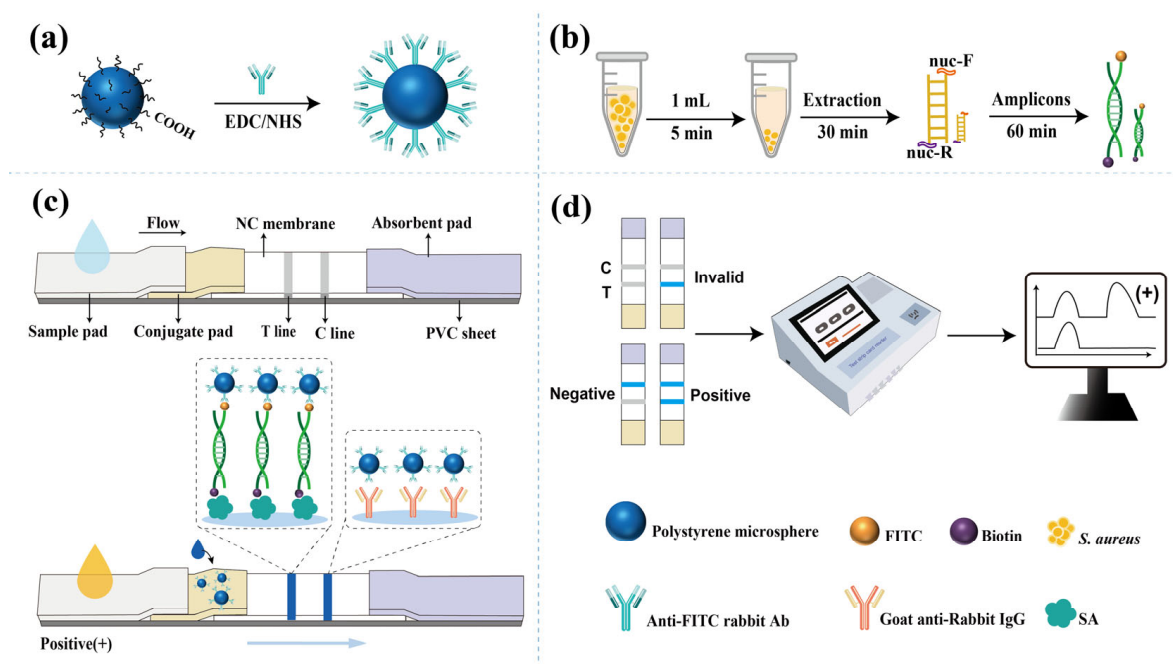


Figure 1. Schematic diagram of the polystyrene microsphere-based lateral flow assay for *Staphylococcus aureus* detection. (a) Preparation of polystyrene microsphere–FITC antibody conjugate; (b) PCR amplification of specific fragments; (c) principle of the polystyrene microsphere-based LFA for *Staphylococcus aureus* detection; (d) quantitative detection by strip reader.

During the detection process, the polystyrene microsphere–FITC antibody complex is pre-dropped onto the conjugate pad. When the sample buffer containing PCR amplification products is added to the sample pad, it migrates towards the absorbing pad under capillary action. The FITC group at one end of the amplified product binds to the conjugate, forming a polystyrene microsphere–FITC antibody–FITC–dsDNA–biotin complex, which continues to migrate with the running buffer. When the complex reaches the T line, the biotin group at the other end of the product is captured by streptavidin fixed on the T line, forming a

sandwich structure and aggregating at the T line for color development (blue). The free conjugate that does not bind to the amplification product continues to migrate forward to the C line and is captured by goat anti-rabbit IgG, forming a second blue band. The standard for interpreting positive results is the simultaneous color development of both T line and C line. The negative result indicates only C line color development. If the C line does not show color, it is determined that the detection is invalid. The color depth of the T line is positively correlated with the concentration of PCR products and can be quantitatively analyzed using a strip reader.

The advantage of this double-label sandwich design lies in the following: (1) the covalent coupling strategy of polystyrene microspheres ensures the stability of the probe in complex sample matrices; and (2) there is no need to denature double-stranded DNA, simplifying the operation process.

3.2. Characterization of AuNPs and Polystyrene Microspheres

The TEM characterization results showed that both AuNPs and polystyrene microspheres exhibited good monodispersity. The AuNPs were spherical in shape with an average diameter of ~20 nm (Figure 2a). To provide a conventional and stable benchmark for LFA comparison, ~20 nm AuNPs were used due to their good colloidal stability and reliable migration behavior on nitrocellulose membranes [50–52]. Polystyrene microspheres showed uniform size distribution, smooth surfaces, clear contours, and an average diameter of ~100 nm (Figure 2b,c). No obvious morphological defects were observed for either nanoparticle type, indicating good stability and suitability for subsequent experiments.

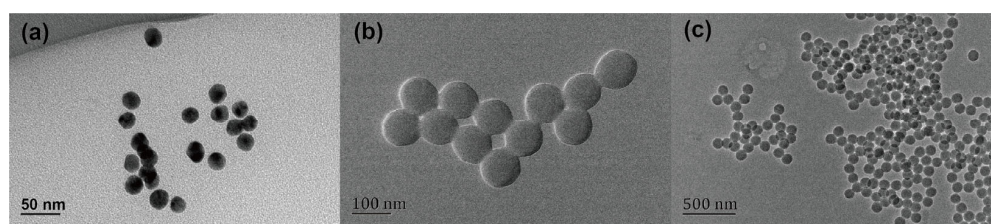


Figure 2. Representative TEM images of AuNPs and polystyrene microspheres. (a) TEM image of citrate-reduced AuNPs; (b,c) TEM images of polystyrene microspheres.

3.3. Optimization of PCR Reaction Conditions

PCR efficiency and non-specific amplification are key factors that affect PCR results. Amplification efficiency directly impacts detection sensitivity, while non-specific amplification, particularly the formation of primer dimers, can lead to false-positive results. To optimize PCR performance, three critical factors were adjusted: magnesium ion concentration, primer concentration, and annealing temperature. This optimization aims to improve amplification efficiency and reduce non-specific amplification, thereby enhancing the reliability and sensitivity of the PCR reaction.

Magnesium ion concentration plays a critical role in influencing the activity of Taq DNA polymerase and, consequently, the efficiency of PCR amplification. In this study, four magnesium ion concentrations—2.5, 3, 3.5, and 4 mM—were evaluated. Agarose gel electrophoresis results (Figure S1a) revealed a unique target band at 279 bp in all four lanes. The intensity of the band in lane 1 was notably weaker, while lanes 2 (with 3 mM Mg^{2+}) and higher showed comparable band intensities. Thus, a final magnesium concentration of 3 mM was selected as the optimal condition for subsequent PCR reactions.

The concentration of primers is another key factor that affects PCR specificity and amplification efficiency. Both excessively high and low primer concentrations can lead to suboptimal results. To optimize the primer concentration, four different final concentrations were tested: 0.5, 1, 1.5, and 2 μ M. Agarose gel results (Figure S1b) showed that as the primer

concentration increased, the target band at 279 bp became progressively more intense, with the clearest band observed at 1 μ M. This concentration was selected as optimal for PCR amplification.

The annealing temperature plays a crucial role in the binding efficiency of primers to the DNA template. If the temperature is too low, primer–template binding may be inefficient, while a high temperature can hinder effective binding. To determine the optimal annealing temperature, a gradient of nine temperatures, ranging from 50 $^{\circ}$ C to 60 $^{\circ}$ C, was tested. Agarose gel results (Figure S1c) indicated that the most distinct amplification bands were observed at 52 $^{\circ}$ C (lane 3), with subsequent temperature increases leading to fainter bands and decreased amplification efficiency. No target bands were observed at temperatures of 56.8 $^{\circ}$ C or higher (lanes 7 and above). Therefore, 52 $^{\circ}$ C was chosen as the optimal annealing temperature.

Finally, the amount of DNA template is a key factor affecting PCR amplification efficiency. Five different DNA template amounts—0.5, 1, 1.5, 2, and 2.5 μ L—were tested. Agarose gel results showed the target band at 279 bp in all lanes, with the most distinct band observed at a template amount of 1.5 μ L. Therefore, 1.5 μ L was selected as the optimal DNA template volume for the standard PCR reaction.

3.4. Optimization of Polystyrene Microsphere-Based PCR-LFA

To optimize the detection performance, key parameters including the concentration of polystyrene microspheres, FITC antibody conjugation, streptavidin concentration, and running buffer were systematically optimized (Figure 3). Specifically, the microsphere amount was optimized by testing 2, 3, 4 and 5 mg/mL of polystyrene microspheres, and the FITC antibody coupling condition was optimized using final antibody concentrations of 30, 40, 50, and 60 μ g/mL. In addition, the SA concentration on the T line was optimized by comparing 0.5, 1.0, and 1.5 mg/mL SA, and the running buffer composition was further optimized using 0.01 M PBS supplemented with 0.1% PVP, 0.5% Tween-20, or 2% BSA.

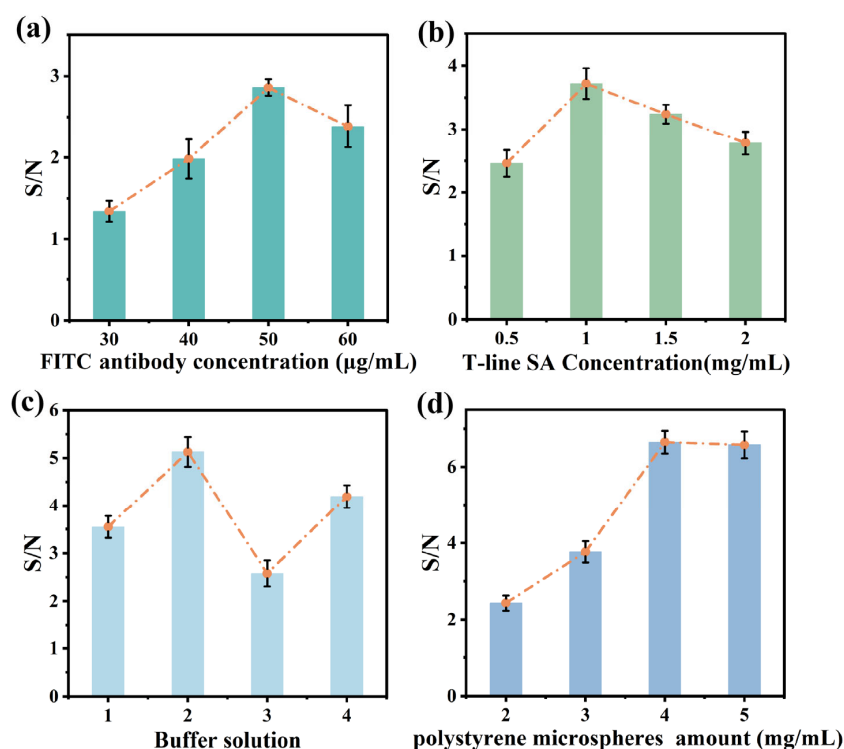


Figure 3. Optimization of the polystyrene microsphere-labeled lateral LFA for *Staphylococcus aureus* detection. Effects of (a) the final concentration of FITC antibody in the microsphere–antibody conjugate;

(b) the streptavidin (SA) concentration on the test line (T line); (c) the running buffer composition (1–4: 0.01 M PBS + 2% BSA, 0.01 M PBS + 0.1% PVP, 0.01 M PBS, and 0.01 M PBS + 0.5% Tween-20), and (d) the concentration of polystyrene microspheres added in the microsphere–antibody conjugate. The signal-to-noise ratio (S/N) was used as the evaluation metric to determine the optimal conditions.

The results indicated that the optimal conditions were 4 mg/mL of polystyrene microspheres, FITC antibody at 50 µg/mL for conjugation, 1 mg/mL SA for the T line, and running buffer consisting of 0.01 M PBS with 0.1% PVP. These optimized conditions effectively improved the sensitivity, specificity, and stability of the strip, ensuring optimal detection performance. Similarly, the detection conditions of AuNP-labeled PCR-LFA were optimized (Figure S2).

3.5. Analytical Performance of the Polystyrene Microsphere-Based PCR-LFA

To comprehensively evaluate the analytical performance of the two PCR-LFA formats, the specificity and sensitivity of the polystyrene microsphere-labeled strip and the AuNP-labeled strip for the detection of *Staphylococcus aureus* were comparatively assessed under optimized conditions (Figure 4a). Under the optimized PCR conditions, PCR amplification was performed on *Staphylococcus aureus* and five other bacterial strains, followed by detection using the optimized PCR-LFS strips. The results showed that only the *Staphylococcus aureus* samples produced a significant positive signal at the T line (Figure 4d), while no noticeable signals were detected for the other five bacterial strains (*Shigella flexneri*, *Shigella sonnei*, *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas putida*). The net grayscale values at the T line showed that the signal of *Staphylococcus aureus* was significantly stronger than that of the other bacterial strains, indicating the high specificity of this method (Figure 4e). Additionally, the performance of the traditional AuNP-labeled strips (Figure 4b,c) was also assessed on the same group of interfering bacteria for comparison, further supporting the specificity of the proposed assay design.

The gradient concentrations of *Staphylococcus aureus* standard bacterial suspension (ranging from 5.6×10^6 to 5.6×10^1 CFU/mL), along with a blank sample, were tested by adding 1.5 µL of DNA template from each concentration level for PCR amplification, followed by detection using the lateral flow strips. The results indicate that a distinct band was observed at a target concentration of 5.6×10^3 CFU/mL (Figure 5d). Data analysis obtained via the strip reader revealed a clear concentration-dependent response (Figure 5e,f). Based on the calibration curve (Figure 5f), the detection limit (LOD) of the method was calculated using the formula $\text{LOD} = 3\sigma/k$ (σ : the standard deviation of the response value of the blank sample; k : the slope of the calibration curve), yielding a LOD of 7.28×10^2 CFU/mL. This detection limit is superior to that of traditional AuNP-labeled strips (LOD: 6.75×10^3 CFU/mL), suggesting that polystyrene microsphere-labeled test strips exhibit higher sensitivity in bacterial detection. The reason why polystyrene microsphere-labeled strips have higher sensitivity might be due to their larger particle size, which can bind more antibody molecules and make low-concentration targets easier to detect. In addition, the optical properties of latex particles (such as high scattering property) can enhance the color development intensity and increase the detection limit.

Subsequently, the reproducibility and stability of the strips were assessed. To evaluate reproducibility, *Staphylococcus aureus* DNA at various concentrations (5.6×10^6 to 5.6×10^3 CFU/mL) was tested in six replicate experiments. By comparing the T-peak area signal values from each experiment, the relative standard deviations of signal intensity were 4.2%, 3.6%, 2.7%, and 1.2%, respectively, demonstrating excellent reproducibility of the strips (Figure S3a). For stability testing, the strips were tested every two months using two different concentrations of *Staphylococcus aureus* DNA (5.6×10^6 CFU/mL, 5.6×10^4 CFU/mL) and blank controls. The results indicated that there was only a slight

signal reduction after six months of storage at room temperature (Figure S3b). These findings confirm that the strips exhibit high stability and reproducibility, making them suitable for field applications.

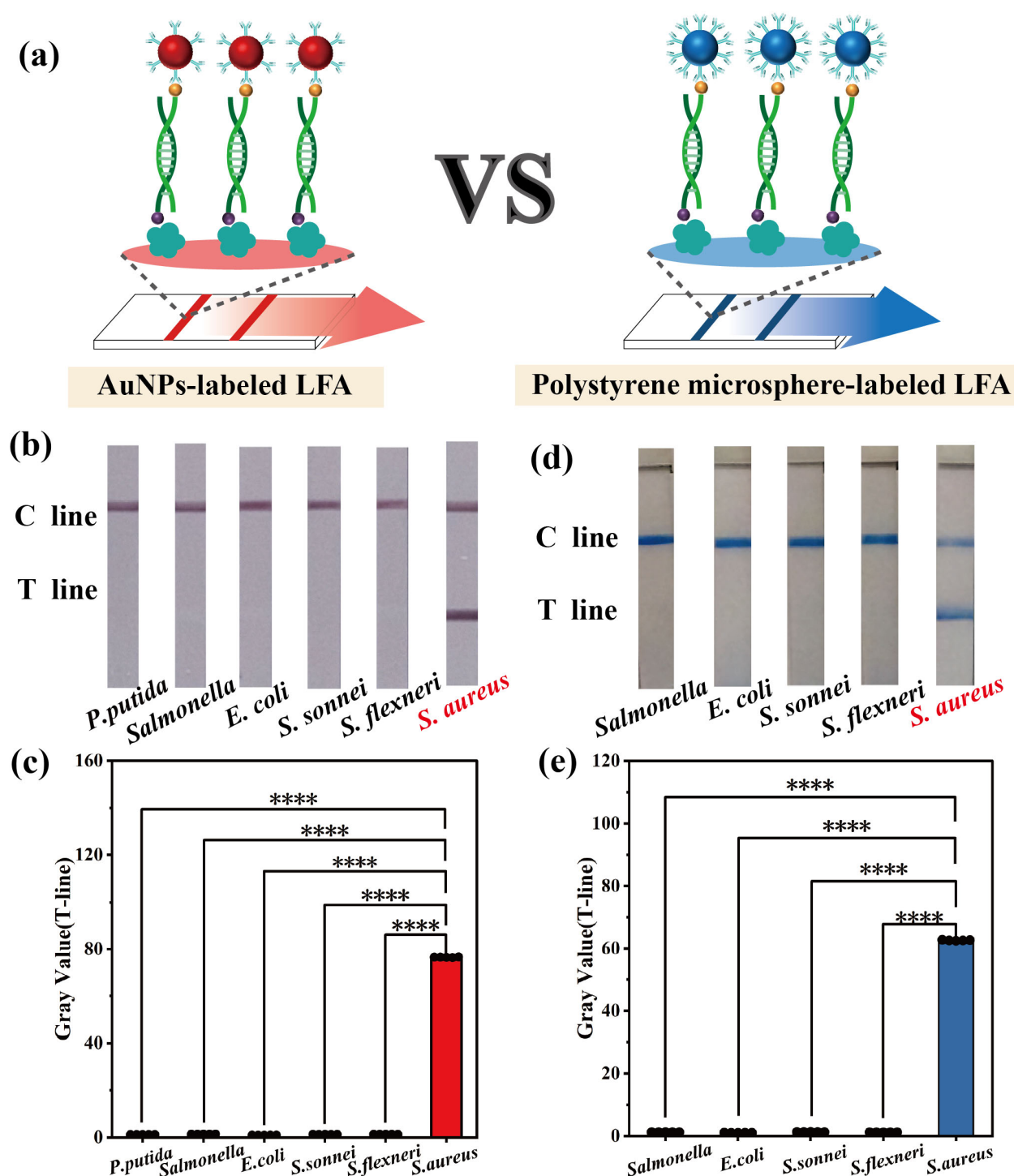


Figure 4. (a) Schematic diagram of the detection principle of AuNP-labeled LFA and polystyrene microsphere-labeled LFA. (b) Images of AuNP-labeled LFA for different bacteria under natural light. (c) Selectivity analysis of AuNP-labeled LFA for *Staphylococcus aureus* detection (bacteria: 5.6×10^6 cfu/mL, **** indicates $p < 0.001$). (d) Images of polystyrene microsphere-labeled LFA for different bacteria under natural light. (e) Selectivity analysis of polystyrene microsphere-labeled LFA for *Staphylococcus aureus* detection (bacteria: 5.6×10^6 cfu/mL, **** indicates $p < 0.001$).

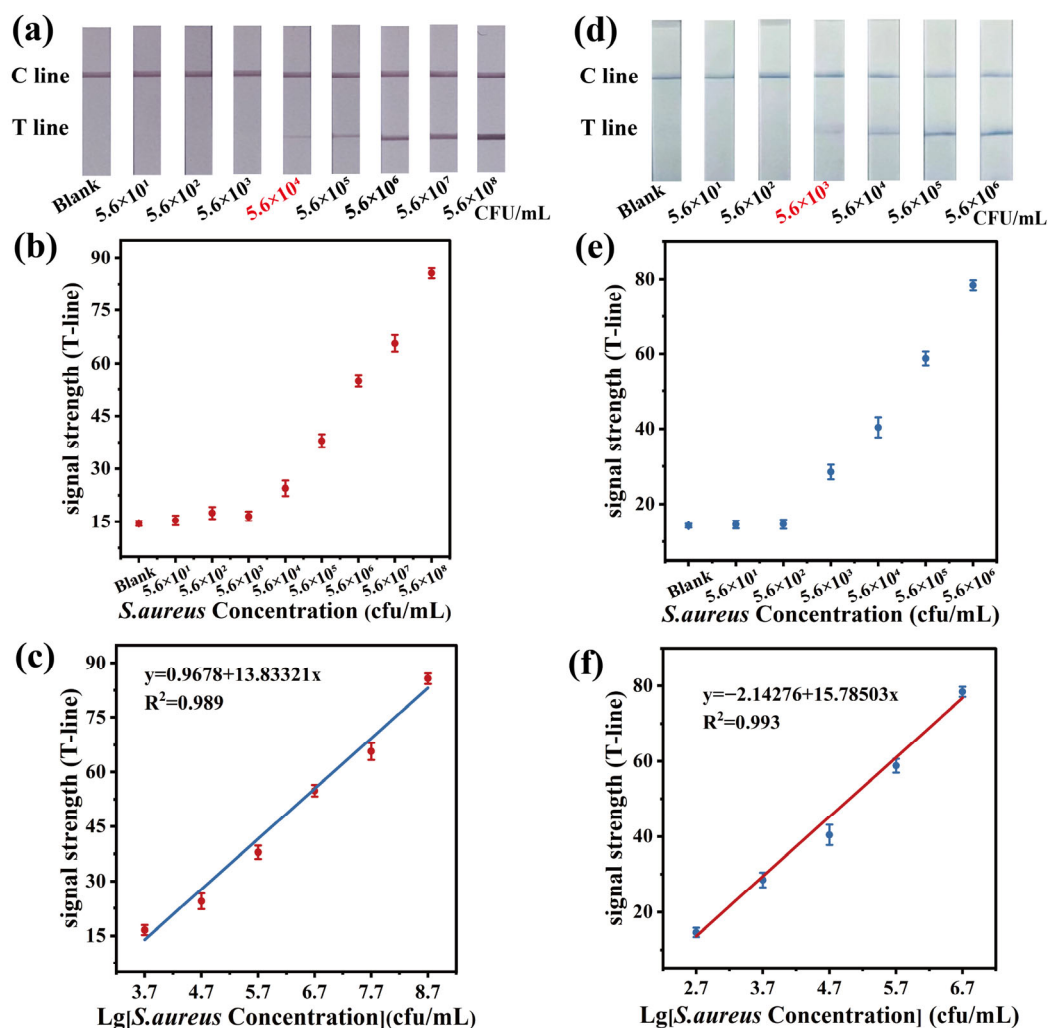


Figure 5. Sensitivity of AuNP-labeled LFA and polystyrene microsphere-labeled LFA for *Staphylococcus aureus* detection. (a) Colorimetric images of AuNP-labeled PCR-LFA for the different concentration of *Staphylococcus aureus* (blank, 5.6×10^1 to 5.6×10^8 CFU/mL). (b) Scatter plots of the gray values at the T line corresponding to different concentrations of bacteria with AuNP-labeled LFA. (c) Linear fitting of the logarithm of the gradient concentration from 5.6×10^3 to 5.6×10^8 (AuNP labeled-LFA). (d) Colorimetric images of polystyrene microsphere-labeled PCR-LFA for the different concentration of *Staphylococcus aureus* (blank, 5.6×10^1 to 5.6×10^6 CFU/mL). (e) Scatter plots of the gray values at the T line corresponding to different concentrations of bacteria with polystyrene microsphere-labeled LFA. (f) Linear fitting of the logarithm of the gradient concentration from 5.6×10^2 to 5.6×10^6 (polystyrene microsphere-labeled LFA).

3.6. Application of the Lateral Flow Strips in Real Samples

To further assess the practical applicability of the developed lateral flow strips in complex food matrices, sterilized milk was selected as a representative sample for *Staphylococcus aureus* detection. No target was detected in the blank samples, demonstrating that the strips exhibit good specificity and compatibility with the background in food matrices. Following this, standard substances were spiked into the blank samples for recovery experiments. First, milk spiked with *Staphylococcus aureus* at varying concentrations (5.6×10^5 – 5.6×10^1 CFU/mL) was tested using polystyrene microsphere-labeled strips, and the results were compared with AuNP-labeled strips (Table S1). This qualitative study revealed that no false positives were observed with the polystyrene microsphere-based strips in the spiked samples, and their sensitivity was found to be superior to that of the AuNP-based strips.

The quantitative detection of *Staphylococcus aureus* in spiked milk samples using the polystyrene microsphere-labeled strips is shown in Table 1. The recovery rates ranged from 96.7% to 104.0%, with standard deviations within an acceptable range. This result indicates that a small amount of matrix components (such as fats, proteins, and vitamins) does not cause significant signal suppression or enhancement, suggesting minimal matrix interference effects and satisfactory quantitative accuracy. These findings collectively demonstrate that the polystyrene microsphere-based strips provide a rapid and reliable method for *Staphylococcus aureus* detection in complex matrices, supporting both qualitative screening and quantitative evaluation.

Table 1. The actual samples assays for *Staphylococcus aureus* via the polystyrene microsphere-labeled strips ($n = 3$).

Sample	<i>Staphylococcus aureus</i> Log (cfu/mL)			Recovery (%)
	Measured	Spiked	Mean $\pm$ SD	
milk	ND ^a	6.0	5.9 $\pm$ 0.2	98.3
	ND	5.0	5.2 $\pm$ 0.2	104.0
	ND	4.0	4.1 $\pm$ 0.3	102.5
	ND	3.0	2.9 $\pm$ 0.1	96.7

^a ND: abbreviation of no detection.

4. Conclusions and Outlook

This study successfully developed a lateral flow assay labeled with polystyrene microspheres for the visual detection of *Staphylococcus aureus* PCR products. Compared with the traditional AuNP antibodies through physical adsorption, carboxylated polystyrene microspheres bind to antibodies through covalent coupling, which has higher stability and signal strength. The results indicate that the visual detection limit for *Staphylococcus aureus* is 5.6×10^3 CFU/mL, which is about 10 times higher than that of AuNP-based strips. After further analysis, the LOD was calculated to reach 7.28×10^2 CFU/mL. The strip exhibits high specificity, excellent reproducibility, and stability. In real-world testing with artificially contaminated milk, this method also demonstrated superior performance compared to the AuNP-based strips. However, the detection effects of more complex food matrices still need to be verified. Future work will further explore multi-detection capabilities and their integration with microfluidic chips and isothermal amplification technologies to build a more complete on-site rapid detection platform.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/bios16020114/s1>, Figure S1: Optimization of PCR reaction conditions. Figure S2: Optimization of AuNP-labeled PCR-LFA for *Staphylococcus aureus* detection. Figure S3: Performance evaluation of polystyrene microsphere-labeled PCR-LFA for *Staphylococcus aureus* detection. Table S1: Comparison of the AuNP-LFA and polystyrene microsphere-LFA in actual samples ($n = 3$, “+” positive, “−” negative).

Author Contributions: Conceptualization, W.Q. and L.Y.; methodology, W.Q. and H.S.; software, L.Z. and J.L.; validation, J.L. and L.L.; formal analysis, L.Z. and L.L.; data curation, Z.Z. and L.Z.; writing—original draft preparation, L.Z. and W.Q.; writing—review and editing, H.S. and L.Y.; supervision, L.Y. and H.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Shanghai Sailing Program (No. 23YF1422800), and the Talent Introduction Project of Anhui Science and Technology University (Grant No. SKYJ202101).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sohrabi, H.; Majidi, M.R.; Khaki, P.; Jahanban-Esfahlan, A.; de la Guardia, M.; Mokhtarzadeh, A. State of the art: Lateral flow assays toward the point-of-care foodborne pathogenic bacteria detection in food samples. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 1868–1912. [\[CrossRef\]](#)
2. Sohrabi, H.; Majidi, M.R.; Fakhraei, M.; Jahanban-Esfahlan, A.; Hejazi, M.; Oroojalian, F.; Baradaran, B.; Tohidast, M.; Guardia, M.d.l.; Mokhtarzadeh, A. Lateral flow assays (LFA) for detection of pathogenic bacteria: A small point-of-care platform for diagnosis of human infectious diseases. *Talanta* **2022**, *243*, 123330. [\[CrossRef\]](#)
3. Tătaru, A.-M.; Canciu, A.; Chiorean, A.-D.; Runcan, I.; Radu, A.; Bordea, M.A.; Suciu, M.; Tertiș, M.; Cernat, A.; Cristea, C. Competitive Electrochemical Apt-Assay Based on cDNA–Ferrocene and MXenes for *Staphylococcus aureus* Surface Protein A Detection. *Biosensors* **2024**, *14*, 636. [\[CrossRef\]](#)
4. Zhou, S.; Wang, Y.; Wang, H.; Dou, S.; Liu, M.; Dong, H.; Li, Z.; Li, D.; Liu, J.; Sun, X.; et al. Rapid detection of *Staphylococcus aureus* in animal-derived foods using aptamer-based fluorescent lateral flow biosensors. *Biochem. Eng. J.* **2025**, *220*, 109756. [\[CrossRef\]](#)
5. Younes, N.; Yassine, H.M.; Kourentzi, K.; Tang, P.; Litvinov, D.; Willson, R.C.; Abu-Raddad, L.J.; Nasrallah, G.K. A review of rapid food safety testing: Using lateral flow assay platform to detect foodborne pathogens. *Crit. Rev. Food Sci. Nutr.* **2023**, *64*, 9910–9932. [\[CrossRef\]](#) [\[PubMed\]](#)
6. McConn, B.R.; Kraft, A.L.; Durso, L.M.; Ibekwe, A.M.; Frye, J.G.; Wells, J.E.; Tobey, E.M.; Ritchie, S.; Williams, C.F.; Cook, K.L.; et al. An analysis of culture-based methods used for the detection and isolation of *Salmonella* spp., *Escherichia coli*, and *Enterococcus* spp. from surface water: A systematic review. *Sci. Total Environ.* **2024**, *927*, 172190. [\[CrossRef\]](#)
7. Guo, Y.; Xu, H.; Wu, Y.; Luo, S.; Hong, Q.; Zhang, X.; Zou, X.; Sun, Z. Conductive MoS₂-Au nanocomposite-based electrochemical biosensor for CRISPR/Cas12a-driven *Staphylococcus aureus* detection. *Sens. Actuators B Chem.* **2025**, *442*, 138078. [\[CrossRef\]](#)
8. Nguyen, T.T.-Q.; Kim, E.R.; Gu, M.B. A new cognate aptamer pair-based sandwich-type electrochemical biosensor for sensitive detection of *Staphylococcus aureus*. *Biosens. Bioelectron.* **2021**, *198*, 113835. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Garbaccio, S.G.; Garro, C.J.; Delgado, F.; Tejada, G.A.; Eirin, M.E.; Huertas, P.S.; Leon, E.A.; Zumárraga, M.J. Enzyme-linked immunosorbent assay as complement of intradermal skin test for the detection of mycobacterium bovis infection in cattle. *Tuberculosis* **2019**, *117*, 56–61. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Yang, Q.; Liu, J.; He, Q.; Zhang, S.; Zhu, L.; Zhang, W.; Han, D.; Xu, W. Recent advances in microfluidic platforms utilizing nucleic acid amplification technology for the detection of foodborne pathogens. *Trends Food Sci. Technol.* **2025**, *165*, 105286. [\[CrossRef\]](#)
11. Jangid, H.; Panchpuri, M.; Dutta, J.; Joshi, H.C.; Paul, M.; Karnwal, A.; Ahmad, A.; Alshammari, M.B.; Hossain, K.; Pant, G.; et al. Nanoparticle-based detection of foodborne pathogens: Addressing matrix challenges, advances, and future perspectives in food safety. *Food Chem. X* **2025**, *29*, 102696. [\[CrossRef\]](#)
12. Aladhadh, M. A Review of Modern Methods for the Detection of Foodborne Pathogens. *Microorganisms* **2023**, *11*, 1111. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Velez, F.J.; Kandula, N.; Blech-Hermoni, Y.; Jackson, C.R.; Bosilevac, J.M.; Singh, P. Digital PCR assay for the specific detection and estimation of *Salmonella* contamination levels in poultry rinse. *Curr. Res. Food Sci.* **2024**, *9*, 100807. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Quintela, I.A.; Vasse, T.; Lin, C.-S.; Wu, V.C.H. Advances, applications, and limitations of portable and rapid detection technologies for routinely encountered foodborne pathogens. *Front. Microbiol.* **2022**, *13*, 1054782. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Huang, L.; Hu, S.; Zheng, Z.; Li, Y.; Xu, M.; Zhang, Z.; Cheng, J.; Zhang, Y.; Xue, Y.; Su, M.; et al. Rapid Detection of *Staphylococcus aureus* from Gym Environments for Health Risk Monitoring Using Printed Nanochains-Based Biosensors. *Biosensors* **2025**, *15*, 791. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Skirda, A.M.; Orlov, A.V.; Malkerov, J.A.; Znoyko, S.L.; Rakitina, A.S.; Nikitin, P.I. Enhanced Analytical Performance in CYFRA 21-1 Detection Using Lateral Flow Assay with Magnetic Bioconjugates: Integration and Comparison of Magnetic and Optical Registration. *Biosensors* **2024**, *14*, 607. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Wei, C.; Wu, A.; Xu, L.; Xu, C.; Liu, L.; Kuang, H.; Xu, X. Recent progress on lateral flow immunoassays in foodborne pathogen detection. *Food Biosci.* **2023**, *52*, 102475. [\[CrossRef\]](#)
18. Wu, P.C.; Song, J.; Sun, C.X.; Zuo, W.C.; Dai, J.J.; Ju, Y.M. Recent advances of lateral flow immunoassay for bacterial detection: Capture-antibody-independent strategies and high-sensitivity detection technologies. *TrAC Trends Anal. Chem.* **2023**, *166*, 117203.
19. Hou, S.; Lin, T.; Ding, Z.; Cui, S.; Cao, Y.; Jiao, J.; Kang, Q.; Du, X. Nanoparticles-based lateral flow immunochromatographic strip for detection of foodborne pathogen: A review. *Food Chem.* **2025**, *496*, 146595. [\[CrossRef\]](#)
20. Yang, M.; Xu, X.; Zhang, M.; Wang, J.; Wu, Y.; Wang, N.; Li, Z. Recent advances in lateral flow immunoassay based on sandwich format for whole-cell pathogen detection. *Coord. Chem. Rev.* **2025**, *533*, 216538. [\[CrossRef\]](#)

21. Chen, P.; Khan, I.M.; Qin, M.; Li, Y.; Raza, A.; Qi, S.; Wang, Z. Advances in lateral flow assays for sensitive detection of antibiotic residues: An intelligent tool for food safety monitoring. *Trends Anal. Chem.* **2025**, *193*, 118462. [\[CrossRef\]](#)
22. Chien, Y.-S.; Tsai, T.-T.; Lin, J.-H.; Chang, C.-C.; Chen, C.-F. One-step copper deposition-induced signal amplification for multiplex bacterial infection diagnosis on a lateral flow immunoassay device. *Biosens. Bioelectron.* **2024**, *267*, 116849. [\[CrossRef\]](#)
23. Han, X.; Fang, R.; Liang, Y.; Hu, P.; Pang, J.; Shen, Y.; Yang, J.; Wen, P.; Xu, Z.; Wang, H. Breakthrough of the lateral flow assay technology for on-site detection of foodborne pathogenic bacteria: Resonance among the recognition reagents, nanomaterial labels, and multi-technological integration. *Food Chem.* **2025**, *493*, 145832. [\[CrossRef\]](#)
24. Silva, G.B.L.; Alvarez, L.A.C.; Campos, F.V.; Guimarães, M.C.C.; Oliveira, J.P. A sensitive gold nanoparticle-based lateral flow immunoassay for quantitative on-site detection of Salmonella in foods. *Microchem. J.* **2024**, *199*, 109952. [\[CrossRef\]](#)
25. Liu, X.; Kukkar, D.; Deng, Z.; Yang, D.; Wang, J.; Kim, K.-H.; Zhang, D. “Lock-and-key” recognizer-encoded lateral flow assays toward foodborne pathogen detection: An overview of their fundamentals and recent advances. *Biosens. Bioelectron.* **2023**, *235*, 115317. [\[CrossRef\]](#)
26. Khan, S.; Dasari, V.V.; Paila, B.; Asok, S.; Nshimiyimana, W.; Bhatt, C.S.; Korupalli, C.; Mishra, A.; Suresh, A.K. Next-Generation Theragnostic Gold Nanoparticles: Sustainable Bioengineering Strategies for Enhanced Stability and Biocompatibility. *Coord. Chem. Rev.* **2025**, *543*, 216925. [\[CrossRef\]](#)
27. Esmaeilpour, D.; Zare, E.N.; Hassanpur, M.; Sher, F.; Sillanpää, M. Comparative examination of the chemistry and biology of AI-driven gold NPs in Theranostics: New insights into biosensing, bioimaging, genomics, diagnostics, and therapy. *Nanomater. Nanotechnol. Biol. Med.* **2025**, *67*, 102821. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Rawat, S.; Phogat, P.; Shreya; Chand, B. Advances in nanomaterial-based biosensors: Innovations, challenges, and emerging applications. *Mater. Today Commun.* **2025**, *48*, 113334. [\[CrossRef\]](#)
29. Xu, M.; Zhao, S.; Peng, Y.; Yang, Y. MoS₂ Nanoflower-Based Colorimetric and Photothermal Dual-Mode Lateral Flow Immunoassay for Highly Sensitive Detection of Pathogens. *Biosensors* **2025**, *15*, 661. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Ruiz, G.; Tripathi, K.; Okyem, S.; Driskell, J.D. pH Impacts the Orientation of Antibody Adsorbed onto Gold Nanoparticles. *Bioconjug. Chem.* **2019**, *30*, 1182–1191. [\[CrossRef\]](#)
31. Hu, W.; Wang, S.; Du, Q.; Yang, S.; Wei, X.; Li, X.; Lin, H. Dual-signal enhanced lateral flow immunoassay with nanobody-functionalized magneto fluorescent nanoprobe for multiplexed detection of foodborne pathogens. *Anal. Chim. Acta* **2025**, *1369*, 344360. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Mateos, H.; Oliver, M. Emerging strategies for the formulation of antibody–nanoparticle conjugation in lateral flow immunoassays. *Curr. Opin. Colloid Interface Sci.* **2025**, *80*, 101968. [\[CrossRef\]](#)
33. Yuan, H.; Yong, R.; Yuan, W.; Zhang, Q.; Lim, E.G.; Wang, Y.; Niu, F.; Song, P. Centrifugation-assisted lateral flow assay platform: Enhancing bioassay sensitivity with active flow control. *Microsyst. Nanoeng.* **2025**, *11*, 101. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Ai, Z.; Cai, H.; Liu, C.; Zhao, Y.; Fu, Q.; Fan, N.; Li, Y.; Li, S.; Zhou, S.; Li, C.; et al. Ultrasensitive Bi-Mode Lateral-Flow Assay via UCNP-Based Host-Guest Assembly of Fluorescent-Colorimetric Nanoparticles. *Small* **2025**, *21*, 2410947. [\[CrossRef\]](#)
35. Tian, Y.; Chen, L.; Liu, X.; Chang, Y.; Xia, R.; Zhang, J.; Kong, Y.; Gong, Y.; Li, T.; Wang, G.; et al. Colored Cellulose Nanoparticles with High Stability and Easily Modified Surface for Accurate and Sensitive Multiplex Lateral Flow Assay. *ACS Nano* **2025**, *19*, 4704–4717. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Wang, Z.; Zhao, J.; Xu, X.; Guo, L.; Xu, L.; Sun, M.; Hu, S.; Kuang, H.; Xu, C.; Li, A. An Overview for the Nanoparticles-Based Quantitative Lateral Flow Assay. *Small Methods* **2021**, *6*, 2101143. [\[CrossRef\]](#)
37. Wang, C.; Xiao, R.; Wang, S.; Yang, X.; Bai, Z.; Li, X.; Rong, Z.; Shen, B.; Wang, S. Magnetic quantum dot based lateral flow assay biosensor for multiplex and sensitive detection of protein toxins in food samples. *Biosens. Bioelectron.* **2019**, *146*, 111754. [\[CrossRef\]](#)
38. Zhou, B.; Ye, Q.; Li, F.; Xiang, X.; Shang, Y.; Wang, C.; Shao, Y.; Xue, L.; Zhang, J.; Wang, J.; et al. CRISPR/Cas12a based fluorescence-enhanced lateral flow biosensor for detection of *Staphylococcus aureus*. *Sens. Actuators B Chem.* **2021**, *351*, 130906. [\[CrossRef\]](#)
39. He, W.; Wang, M.; Cheng, P.; Liu, Y.; You, M. Recent advances of upconversion nanoparticles-based lateral flow assays for point-of-care testing. *Trends Anal. Chem.* **2024**, *173*, 117641. [\[CrossRef\]](#)
40. Liang, Z.; Peng, T.; Jiao, X.; Zhao, Y.; Xie, J.; Jiang, Y.; Meng, B.; Fang, X.; Yu, X.; Dai, X. Latex Microsphere-Based Bicolor Immunochromatography for Qualitative Detection of Neutralizing Antibody against SARS-CoV-2. *Biosensors* **2022**, *12*, 103. [\[CrossRef\]](#)
41. Razo, S.C.; Elovenskova, A.I.; Safenkova, I.V.; Drenova, N.V.; Varitsev, Y.A.; Zherdev, A.V.; Dzantiev, B.B. Comparative Study of Four Coloured Nanoparticle Labels in Lateral Flow Immunoassay. *Nanomaterials* **2021**, *11*, 3277. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Xu, N.; Zhu, Q.; Zhu, J.; Jia, J.; Wei, X.; Wang, Y. Novel Latex Microsphere Immunochromatographic Assay for Rapid Detection of Cadmium Ion in Asparagus. *Foods* **2021**, *11*, 78. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Wang, J.; Li, X.; Shen, X.; Zhang, A.; Liu, J.; Lei, H. Polystyrene Microsphere-Based Immunochromatographic Assay for Detection of Aflatoxin B1 in Maize. *Biosensors* **2021**, *11*, 200. [\[CrossRef\]](#)

44. Huang, Y.; Xu, A.; Xu, Y.; Wu, H.; Sun, M.; Madushika, L.; Wang, R.; Yuan, J.; Wang, S.; Ling, S. Sensitive and rapid detection of tetrodotoxin based on gold nanoflower-and latex microsphere-labeled monoclonal antibodies. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1196043. [[CrossRef](#)]
45. Zang, X.; Zhou, Y.; Li, S.; Shi, G.; Deng, H.; Zang, X.; Cao, J.; Yang, R.; Lin, X.; Deng, H.; et al. Latex microspheres lateral flow immunoassay with smartphone-based device for rapid detection of *Cryptococcus*. *Talanta* **2024**, *284*, 127254. [[CrossRef](#)]
46. Jin, B.; Du, Z.; Zhang, C.; Yu, Z.; Wang, X.; Hu, J.; Li, Z. Eu-Chelate Polystyrene Microsphere-Based Lateral Flow Immunoassay Platform for hs-CRP Detection. *Biosensors* **2022**, *12*, 977. [[CrossRef](#)]
47. Machado, I.; Goikoetxea, G.; Alday, E.; Jiménez, T.; Arias-Moreno, X.; Hernandez, F.J.; Hernandez, L.I. Ultra-Sensitive and Specific Detection of *S. aureus* Bacterial Cultures Using an Oligonucleotide Probe Integrated in a Lateral Flow-Based Device. *Diagnostics* **2021**, *11*, 2022. [[CrossRef](#)] [[PubMed](#)]
48. Srisrattakarn, A.; Tippayawat, P.; Chanawong, A.; Tavichakorntrakool, R.; Daduang, J.; Wonglakorn, L.; Lulitanond, A. Development of a Prototype Lateral Flow Immunoassay for Rapid Detection of Staphylococcal Protein A in Positive Blood Culture Samples. *Diagnostics* **2020**, *10*, 794. [[CrossRef](#)] [[PubMed](#)]
49. Dong, J.; Carpinone, P.L.; Pyrgiotakis, G.; Demokritou, P.; Moudgil, B.M. Synthesis of Precision Gold Nanoparticles Using Turkevich Method. *KONA Powder Part. J.* **2020**, *37*, 224–232. [[CrossRef](#)]
50. Kim, D.; Kim, Y.; Hong, S.; Kim, J.; Heo, N.; Lee, M.-K.; Lee, S.; Kim, B.; Kim, I.; Huh, Y.; et al. Development of Lateral Flow Assay Based on Size-Controlled Gold Nanoparticles for Detection of Hepatitis B Surface Antigen. *Sensors* **2016**, *16*, 2154. [[CrossRef](#)] [[PubMed](#)]
51. Chen, X.; Leng, Y.; Hao, L.; Duan, H.; Yuan, J.; Zhang, W.; Huang, X.; Xiong, Y. Self-assembled colloidal gold superparticles to enhance the sensitivity of lateral flow immunoassays with sandwich format. *Theranostics* **2020**, *10*, 3737–3748. [[CrossRef](#)] [[PubMed](#)]
52. Guliy, O.I.; Dykman, L.A. Gold nanoparticle-based lateral-flow immunochromatographic biosensing assays for the diagnosis of infections. *Biosens. Bioelectron. X* **2024**, *17*, 100457. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.