Nanomed Res J 10(4):333-351, Autumn 2025

REVIEW ARTICLE

Contemporary Advances in Optical Nanoparticle-Enhanced Lateral Flow Immunoassays for Rapid Bacterial Detection

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ARTICLE INFO

Article History:
Received 26 Aug 2025
Accepted 13 Nov 2025
Published 01 Dec 2025

Keywords:
Lateral Flow Immunoassay (LFIA)
Optical Nanoparticles
Gold Nanoparticles (AuNPs)
Point-of-Care Testing (POCT)
Bacterial Pathogen Detection
Biosensing and Diagnostics

ABSTRACT

The advancement of precise diagnostic tools is crucial for the prompt identification of bacterial pathogens and combating the increasing issue of antimicrobial resistance. Lateral Flow Immunoassay (LFIA) has emerged as an effective and promising point-of-care testing (POCT) approach, playing a distinctive role in biomedical sciences, food safety, agriculture and infectious disease diagnostics due to its ease of use, high speed, portability and cost-effectiveness. Utilizing on a paper-based platform and functionalized nanoparticle probes, this technique is capable of identifying a broad spectrum of analytes, such, as whole bacterial cells, nucleic acids, proteins and pathogen-specific biomarkers. Lately, the application of new types of optical nanoparticles, such as quantum dots (QDs) and upconversion nanoparticles (UCNPs) has dramatically improved the sensitivity, specificity and signal resolution of LFIA-based diagnostic strips. This review article outlines the fundamental principles and key components of LFIAs, highlights recent progress in the design and types of nanoparticles, and performance optimization of these systems for the rapid and specific detection of pathogenic bacteria. Finally, it discusses current challenges and future prospects of this technology in the field of clinical diagnostics and point-of-care analysis.

How to cite this article
Safari M., Salek maghsoudi A., Sadeghzadeh M., Mirmotahari S.A., Hassani Sh., Amini M. Contemporary Advances in Optical Nanoparticle-Enhanced Lateral Flow Immunoassays for Rapid Bacterial Detection. Nanomed Res J, 2025; 10(4): 333-351.
DOI: 10.22034/nmrj.2025.04.002

INTRODUCTION

Bacteria are microbes that are able to live and multiply in diverse environmental settings. Although numerous bacteria contribute positively to functions, certain species are pathogenic and can rapidly multiply, leading to various infections, including foodborne diseases and serious systemic conditions like septicemia and pneumonia[1, 2]. Serious complications and death can be caused by delays in diagnosis and treatment. Therefore, the rapid detection of bacterial infections is both a treatment imperative and a critical factor in halting the transmission of infectious illnesses. Increased antimicrobial resistance (AMR) and prolonged patient hospitalization in clinical settings, are serious outcomes of delays in pathogen identification[3, 4].Rapid and precise point-of-care (POC) diagnostic tools are becoming essential for managing infections that cause major health and financial challenges globally, especially in areas with limited resources[5].

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Traditional diagnostic approaches, including culture, Gram staining and molecular techniques such as polymerase chain reaction (PCR), while very sensitive and dependable, usually require sophisticated instruments, extended processing durations and skilled operators, making them impractical for quick or field-based testing[6 ,7]. As a result, the development of sensitive, affordable, and easy-to-use diagnostic tools has garnered considerable interest as a practical substitute for traditional methods like ELISA and PCR. Among diagnostic innovations, biosensors have quickly progressed because they enable qualitative and semi-quantitative pathogen detection without requiring complicated procedures. Lateral flow immunoassay (LFIA) stands out as one of the leading platforms, for diagnostic purposes. These tests provide benefits including straightforwardness, affordability, user-friendliness, and the capability to provide visually interpretable results, which makes them some of the most commonly used POC diagnostic tools. A key application of LFIA technology lies in disease detection, as these tests can selectively recognize pathogen-related biomarkers, like antigens, proteins, and other target molecules. The principal mechanism of LFIAs is based on biochemical interactions between antigen-antibody binding and probe-target DNA hybridization [5-7]. Although LFIAs offer several benefits, they continue to encounter issues with sensitivity, particularly in identifying low bacterial concentrations[7]. To overcome this limitation, nanoparticles have revolutionized the LFIA platform by acting as effective optical signal generators. Gold nanoparticles (AuNPs) are regarded as the classic label[8], but recent progress has emphasized integrating more sophisticated optical nanoparticles, including quantum dots (QDs)[9], upconversion nanoparticles (UCNPs) and carbon-based nanoparticles (CNPs)[10]. These nanoparticles demonstrate characteristics, such as high stability, adjustable optoelectronic features, biocompatibility, and the capacity to generate strong visual signals, which facilitate substantial signal enhancement, reduced limits of detection (LOD), and even allow for multiplex pathogen detection. Nevertheless, practical and technical obstacles still need to be resolved [11, 12]. This comprehensive review aims to analyze the current state of research in the field of optical nanoparticle-enhanced lateral flow immunoassay (LFIA) for bacterial detection. The primary focus is to highlight the integration of optical nanoparticles to enhance sensitivity and enable the multiplexed detection of bacterial pathogens. Following an overview of the principles, structure, and components of LFIA technology and its application in bacterial detection. Finally, the review addresses the existing challenges, current limitations, and future perspectives in advancing this promising diagnostic technology.

LATERAL FLOW IMMUNOASSAY

Principle of LFIA

Lateral flow immunoassay (LFIA) originated in the 1960s as one of the rapid diagnostic methods designed to track serum proteins. In 1976, these assays were first practically applied to identify chorionic gonadotropin (HCG) in the urine samples. The working mechanism of LFIA relies on antigen–antibody binding, allowing for targeted analyte detection, with high selectivity. LFIA has been widely employed for identifying a variety of substances, such, as pesticides, cancer markers, mycotoxins, microbes, and heavy metals. In scientific and industrial literature, this technology is also referred as the Lateral Flow Device (LFD), Dipstick Test, Rapid Test, Point-of-Care Test (POCT) and Pen-side Test. The test operates on the principle of the capillary flow of a liquid sample or fluid containing the analyte across a test strip (Figure. 1). This strip is usually made from polymeric materials, segmented into separate zones where biorecognition molecules are conjugated with specific labels. As the sample flows through these segments, the target analyte binds specifically to immobilized capture molecules, forming detectable antigen–antibody complexes. The specimen moves across the membrane via capillary force, and the resulting signal typically a visible colored line shows whether the target analyte is present or not. LFIA combines flow chromatography with immunochemical reactions, allowing specific identification, via antigen–antibody interactions. The assay delivers qualitative results, or in some cases, quantitative data when coupled with analytical software or optical readers[13, 14].

Initially, the liquid sample is applied to the sample pad, which ensures uniform distribution and directs the flow toward the conjugate pad. The conjugate pad, contains preloaded antibodies conjugated to colored nanoparticles, typically gold nanoparticles (AuNPs). When the target analyte is present, it attaches to these conjugates, creating

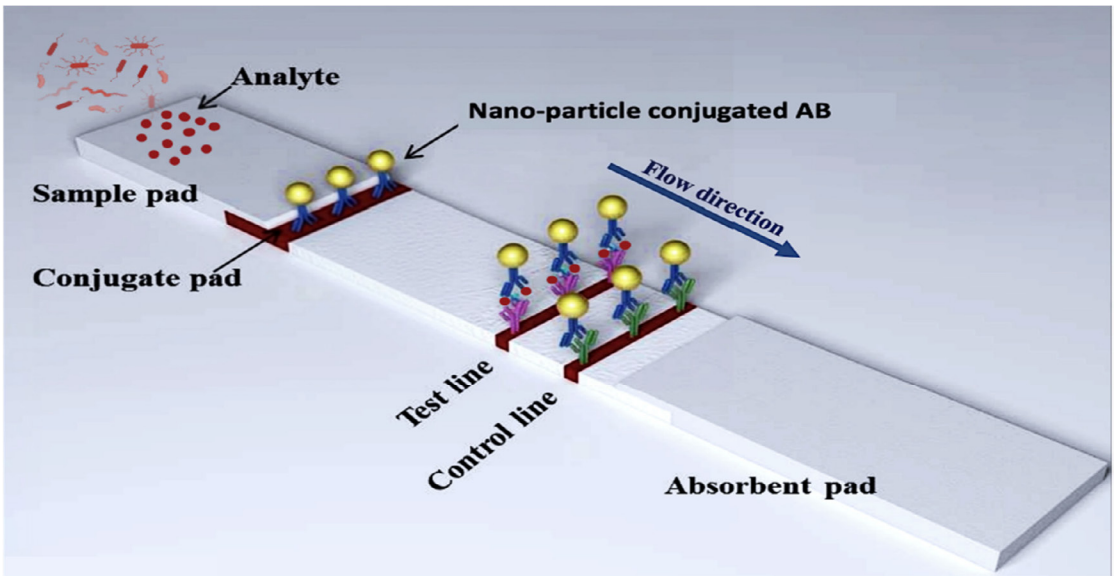

Fig.1. Schematic of a lateral flow immunoassay for bacterial detection.

antigen–antibody–nanoparticle complexes, that then move onto the nitrocellulose membrane. This membrane has two areas: the test line (TL) and the control line (CL), where antigen or antibody molecules are immobilized. At the test line, the accumulation of these complexes produces a colored band, the intensity correlates with the analyte concentration and the type of nano label applied. The presence of bands on both the test and control lines indicates a positive result, whereas a single band solely at the control line indicates a negative result. The absence of a band at both lines, or at the control line alone, is considered an invalid result[15, 16]. Gold nanoparticles (AuNPs) are the most commonly used due to their ease of surface functionalization, adjustable particle size, and strong surface plasmon resonance (SPR) properties, which produce vivid and visible colorimetric signals. The distinctive optical characteristics of AuNPs enable observation of antigen–antibody reactions with the signal strength quantitatively reflecting the analyte concentration [17]. The sensitivity and specificity of LFIA depends on several factors, such as antibody affinity, nanoparticle physicochemical properties, and the strip's design. The use of high-affinity antibodies enhances target-binding efficiency, while optimizing nanoparticle size, membrane porosity, and flow rate enhances signal strength and reduces detection limits. Collectively, these factors enhance assay effectiveness and ensure reliable detection of bacterial pathogens, viruses, and other analytes, even at low concentrations[5]. The use of quantum dots (QDs), upconverter nanoparticles (UCNPs), and other advanced optical nanomaterials have opened new avenues in biomarker detection[18, 19].

Components of the LFIA Strip

The lateral flow immunoassay (LFIA) strip is an analytical membrane-based platform comprising four principal components: the sample pad, conjugate pad, nitrocellulose membrane and absorbent pad which are integrated onto a rigid backing card to form a user-friendly structure. Each of these elements is precisely engineered to facilitate capillary-driven fluid flow, enable specific recognition of the target analyte, and generate a detectable signal, collectively determining the assay's sensitivity, specificity, and operational robustness[20].

Sample Pad

This pad serves as the initial interface for the sample application. It is typically composed of cellulose acetate or glass fiber materials. Its selection is based on its capacity to retain buffers, surfactants, and stabilizing proteins to facilitate uniform liquid flow during sample application, while also ensuring homogeneous fluid delivery to downstream components. The application

of this pad is usually to remove interfering biomolecules and reduce non-specific antigen-antibody interactions, thereby improving analytical reproducibility. Pre-treatment of the sample pad is crucial for enhancing analytical performance by increasing sample viscosity, preventing overflow, and regulating the liquid's flow rate toward the conjugate pad[5, 21].

Conjugate Pad

This pad contains lyophilized bioreagents, typically antibodies or aptamers conjugated to nanoparticles, which are rehydrated upon contact with the migrating liquid sample. Following hydration, these conjugates selectively bind the analyte to form labeled antigen–antibody complexes that migrate toward the nitrocellulose membrane. To ensure optimal release and stability, the conjugate pad (often made of glass fiber, cellulose, or polyester) is pre-treated with salt solutions, surfactants, stabilizers, and blocking agents[22].

Nitrocellulose Membrane

The NC membrane acts as the detection center of the LFIAs strip with the test line (TL) and control line (CL) affixed to trap analyte–complexes and produce a visible signal. The TL is tailored for the identification of the target analyte, whereas the CL serves as an internal check to verify the assay is working correctly. Key parameters, including pore size (ranging from 0.05 to 12 μm), capillary flow rate, protein-binding capacity, and membrane uniformity, critically influence assay reproducibility, signal intensity, and the limit of detection (LOD). Despite the availability of alternative polymers, nitrocellulose remains the preferred substrate due to its high protein affinity, easy surface modification and cost efficiency[23].

Absorbent Pad

The absorbent pad, also referred to as the wicking pad, is positioned at the terminal end of the strip and acts as a capillary sink, maintaining continuous fluid flow while preventing undesirable backflow. Composed primarily of high-density cellulose, it governs the hydrodynamic balance of the system, ensuring complete reagent migration across the strip. Sequential overlap of all pads on the backing card ensures structural integrity, seamless fluid migration, and mechanical protection of the delicate NC membrane[24].

Backing Card

All LFIA components are laminated onto a backing card, which maintains precise alignment and mechanical support for each pad following reagent deposition. This configuration facilitates handling and reproducibility during large-scale manufacturing. Depending on the intended diagnostic format, the strip can be arranged in a simple dipstick or cassette-based[25].

Classification of LFIA Techniques

LFIA systems can be categorized according to the variety of analytes, detection mechanisms, or assay designs, demonstrating their adaptability, for numerous diagnostic uses. Typically, LFIA falls into three groups: antibody-based, antigen-based, and nucleic acid-based formats, each optimized for specific molecular targets[26]. Antibody-based LFIA represents the widely applied format, mainly employed to identify proteins, pathogens, and small compounds. In this setup, antibodies act as both the capture and detection reagents, providing high specificity for the target analyte. Based on the assay configuration, two primary subtypes are utilized: the sandwich format, suitable for larger analytes with multiple epitopes, and the competitive (inhibition) format, typically used for small molecules where steric hindrance blocks the concurrent binding of two antibodies[27, 28]. Antigen-based LFIA is designed for the identification of particular antigens or elements originating from pathogens. In this format, antibodies fixed on the test line bind the target antigen from the applied sample, while labeled detection antibodies produce a visible signal. This arrangement is commonly used for infectious disease testing, allowing the identification of bacterial or viral proteins in clinical samples [29, 30]. Nucleic acid-based LFIA utilizes labeled oligonucleotide probes to identify specific DNA or RNA sequences. These tests are frequently combined with isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA) to provide rapid, sensitive, and highly accurate detection of genetic targets. Such assays have become indispensable tools in molecular diagnostics, pathogen surveillance and genotyping studies, owing to their rapid turnaround times and low detection limits[31, 32]. Apart from the analyte category, LFIA can be classified according to its labeling approaches. Colorimetric LFIA, which

employs gold nanoparticles (AuNPs) or colored latex beads, generates visually interpretable results and is especially apt for point-of-care testing (POCT). In contrast, fluorescent, chemiluminescent, or magnetic particle-based LFIA provides sensitivity and allows for quantification when combined with suitable detection technologies [33, 34]. The two primary assay formats in LFIA are the sandwich format and the competitive format.

Sandwich Format

This format is typically applied to analytes possessing multiple epitopes. Two complementary antibodies recognize the analyte simultaneously. One epitope attaches to a labeled antibody (for instance conjugated to gold nanoparticles), known as the detection antibody, forming an antigen–antibody–nanoparticle complex. This complex moves along the membrane and attaches to the capture antibody, immobilized at the test line, leading to the formation of a "sandwich" configuration. The accumulation of labeled complexes produces a visible colored line that can be detected visually or quantitatively using optical or magnetic readers, depending on the labeling material. Excess detection antibodies bind to the control line, typically containing species-specific anti-IgG antibodies, confirming proper assay performance. The simultaneous appearance of both test and control lines indicates the presence of the target analyte, whereas the presence of only the control line confirms a negative result[35, 36].

Competitive Format

This format is mainly employed to identify molecules or haptens that have a single antigenic epitope. In this setup, the signal output strength is inversely related to the analyte concentration. The labeled analyte–antibody complex is pre-immobilized on the conjugate pad, while the test line contains pre-coated antigen molecules. As the sample moves along, free analyte molecules in the sample compete with the immobilized antigen for binding to the labeled antibody. Consequently, at higher analyte concentrations, fewer labeled antibodies bind to the test line, resulting in a weaker signal. The control line, coated with secondary antibodies specific to the labeled conjugate, serves as an internal quality control. Therefore, in this format, increasing analyte concentration leads to a decrease in signal intensity—opposite to the trend observed in the sandwich format [37, 38].

Application of LFIA in Bacterial Detection

Recently identifying infections and their associated toxins through LFIA has become a crucial method for delivering rapid, precise, and sensitive outcomes directly at the point-of-care (POC). This approach is essential for bedside diagnosis and monitoring, in public health because it offers fast results, does not require complicated devices or expert staff, and is easily transportable. Recent progress has concentrated on improving sensitivity and specificity, along with allowing outcomes via incorporation of sophisticated technologies. In response to the growing need for rapid and cost-effective diagnostic methods, extensive research in recent years has focused on developing LFIA for detecting various Gram-positive and Gram-negative bacteria. A brief overview of its application follows, and subsequently, a table summarizing the diverse uses of this technique for detecting different bacteria from 2013 to 2025 is provided (Table 1).

Detection of Gram-Negative Bacteria

So far, LFIA has been used to detect members of the Enterobacteriaceae family, particularly Escherichia coli, Salmonella, and Shigella species, among gram-negative bacteria. These enteric bacteria, possessing endotoxins and exotoxins, are significant causal agents of waterborne and foodborne diseases.

Escherichia coli

This bacterium, the serotype *Escherichia coli* O157:H7—which is responsible for hemorrhagic colitis and hemolytic uremic syndrome—has been widely identified using LFIAs. One investigation utilized 40 nm colloidal gold nanoparticles conjugated with a mouse monoclonal antibody against lipopolysaccharide (LPS), achieving a limit of detection (LOD) of 1.8×10^5 CFU/mL[39]. The use of immunomagnetic separation (IMS) improved this detection threshold to 10^3 CFU/mL, with 95.5% sensitivity and 100% specificity[40]. Innovative techniques, like aptamer-based magnetic separation, have greatly enhanced sensitivity down to 10 CFU/mL. These methods utilize two different aptamers that bind to distinct outer membrane proteins[41]. Previous research has documented the identification of this pathogen in water and milk samples at concentrations of 100 CFU/mL through a fluorescence approach involving quantum dots combined with graphene

oxide (GO) sheets[42]. This serotype has been identified in food samples, such as powdered milk and flour, with a comparable LOD of 10^5 CFU/mL[43, 44]. In a study by Cam et al. the detection of *E. coli* in contaminated food and water was reported using a gold nanoparticle-based lateral flow assay, achieving a visual limit of detection (LOD) in the range of 10^5 CFU/mL within 3 to 5 minutes[45].

Salmonella

Conventional LFIA has been reported for the detection of *Salmonella* with a detection limit of 10^7 CFU/mL[46], and for the specific detection of *Salmonella enteritidis* with a detection limit of 10^4 CFU/mL[46]. However, the integration of LFIA with aptamers has enhanced the sensitivity [47]. Bu et al. developed an LFIA for detecting *Salmonella enteritidis* in milk samples, reporting a detection limit of 10^4 CFU/mL within a total assay time of 6 hours, which demonstrated higher sensitivity compared to conventional LFIAs[48].

Detection of Other Species

LFIA has also been successfully applied for the detection of other bacterial species. Reported detection limits include 10^7–10^8 CFU/mL for *Vibrio cholerae*[49], 10^2 CFU/mL for *Enterobacter*[8], and various targets for *Campylobacter*. Specifically for *Campylobacter jejuni*, a 15 kDa surface protein was targeted in an LFIA, enabling detection with a sensitivity of 84.8% and a specificity of 100% in less than 15 minutes[50].

Detection of Gram-Positive Bacteria
Staphylococcus aureus

This bacterium, which is a major cause of food poisoning in processed foods such as red meat and fried chicken, has been detected using the sandwich LFIA format with 100% specificity[6, 51].

Bacillus anthracis

Bacillus anthracis, which causes anthrax is a biothreat and the urgency for swift identification of anthrax spores has gained focus from scientists because of their deployment, in bioterrorism incidents. Combining LFIAs with immunomagnetic separation (IMS) has allowed for identifying bacterial spores in water and dairy products, with detection thresholds reported between 10^5 to 10^7 CFU/mL[52]. Among other Gram-positive bacteria, the detection of *Streptococcus suis* has been reported with a detection limit of 10^6 CFU/mL[53] and *Listeria monocytogenes* has a detection limit of 3.7×10^6 CFU/mL in spiked milk samples within 13 hours has been reported[54].

Detection of Bacterial Toxins
Staphylococcal Enterotoxin B (SEB)

This toxin has been detected using 25 nm colloidal gold nanoparticles, achieving a detection limit of 1 ng/mL in less than 5 minutes[55]. The use of fluorescent immunoliposomes enhanced this sensitivity to 20 pg/mL[56], while the application of SERS enhancement using gold nanospheres conjugated with the MGITC reporter molecule further improved the detection limit to 0.001 ng/mL[57].

Botulinum Neurotoxin (BoNT)

A sandwich LFIA using gold nanoparticles has been reported for the detection of BoNT /D, with a detection limit of 50 pg/mL[58]. For BoNT/A, a monoclonal antibody conjugated to gold nanoparticles with silver signal enhancement was used, achieving a detection limit of 1 ng/mL without cross-reactivity against BoNT/B or BoNT/E. Furthermore, the simultaneous detection of BoNT/A and BoNT/B with specific antibodies for each target was also possible[59].

Optical Nanoparticle Labels Used in LFIA

Optical nanoparticles are essential elements in lateral flow immunoassays (LFA), facilitating both qualitative and quantitative detection. These nanoparticles are of particular importance because they directly affect key parameters such as specificity, sensitivity, and detection limit of rapid diagnostic kits. This review focuses on the key optical nanoparticles used in LFA.

Gold Nanoparticles

Gold nanoparticles (Au NPs) are among the most widely utilized nanomaterials in rapid diagnostic kits, owing to their unique physicochemical properties. The most common method for manufacturing gold nanoparticles is the Turkovich method, which results in the production of stable and homogeneous nanoparticles that can be used in various medical applications such as imaging and biosensors[67]. Regarding optical properties, Au NPs possessing characteristics such as strong surface plasmon resonance (SPR) arising from the collective oscillation of free electrons,

Table 1. Overview of LFIA Applications in Bacterial Detection (2013-2025)

Year	Bacterial Target	Nanoparticle Label	Detection Technique	Limit of Detection (LOD)	Sample Matrix	References
2013	*Bacillus anthracis*	Superparamagnetic iron oxide particles	LFIAs/ dual-antibody nanoparticle label	4×10^3-10^6 CFU ml^{-1} (detection limits of 200 spores mg^{-1} milk powder and 130 spores mg^{-1} soil)	Milk powder, soil	[52]
2014	*Salmonella enteritidis*	Au NPs + aptamer-based SDA	Lateral flow biosensor/ aptamer-SDA	10 CFU/mL	Food samples	[60]
2016	Multiple foodborne pathogens	Up-converting phosphor technology (UCPs)	Up-converting phosphor technology-based lateral flow (TC-UPT-LF) assay	10^4 CFU mL−1 or 10^5 CFU mL−1 for each pathogen	Food/water	[61]
2018	*Yersinia pestis* (plague)	Up-converting phosphor nanoparticles (UCPs)	LFIAs	10 to 100 folds	Simulated clinical samples such as human serum	[62]
2019	*Escherichia coli* O157:H7	Quantum dots (QDs), Carbon NPs, AuNPs	LFIAs/ Fluorescence	1.1 ± 0.6 nM	Food spiked with E.coli O157:H7	[42]
2019	*Salmonella*	AuNPs + recombinant polymerase amplification (RPA) + LFIAs	LFIAs/ recombinase polymerase amplification (LFD-RPA)	1.29×10^2 CFU/mL	Food deliberately contaminated	[63]
2023	*Vibrio cholera*	Au NPs	RAA-TS-DT[1]	LODs of the gyrB and vvhA genes were 6 CFU/mL and 23 CFU/mL, respectively,	Aquatic products (fish, shrimp and oyster)	[64]
2024	*Burkholderia pseudomallei*	Au NPs	LFIAs/capsular polysaccharide	107 - 106 CFU/mL in hemoculture and artificial urine, respectively.	Hem culture and artificial urine	[65]
2025	*Brucella* spp	Au NPs	LFIA solid phase (multi-laminated membrane strip)	1.58 S/P ratio ELISA titer/100 µl by using LFIA	Animal products	[66]

[1] recombinase-aided amplification (RAA) / double T-lines (RAA-TS-DTL

allow for easy visual detection and enhance the simplicity and accessibility of measurement. The SPR from gold nanoparticles induces strong absorption of intense light, which can be measured using a UV-Vis absorption spectrometer. The SPR band for plasmonic nanoparticles (specifically gold and silver) is significantly more intense than for other metals. The SPR band of gold nanoparticles appears in the visible region at around 520 nm[68]. According to May's theory, the observed SPR intensity depends on factors such as particle size, the type of metal, structure, shape, dielectric constant, and composition, which affect the electron charge density on the particle surface[69]. Since the characteristics of gold nanoparticles, especially their size, directly affect the sensitivity of LFIA, many studies have been conducted to optimize the size of Au NPs. The results show that Au NPs with a size of about 30–40 nm are suitable for use in rapid detection kits because smaller particles have an unacceptable extinction cross-section, while larger particles are unstable after the assay[70]. Additionally, the brightness of a test line is influenced by the number of adsorbed Au NPs and the extinction cross-section of a particle. Khalebtsov et al. prepared and characterized spherical and monodispersed Au nanoparticles of different sizes (from 16 to 115 nm) to assess the relationship between the size of Au nanoparticle and limit of detection (LOD). They applied Au nanoparticles at various dilutions onto the membranes and measured the signal intensity at each spot. The findings of this study indicated the inverse correlation between the size of Au nanoparticle and LOD, where the LOD was proportional to the Au particle size^3.1 for each spot on the surface[71]. In terms of surface chemistry, Au nanoparticles have excellent surface modification capabilities and can be easily functionalized with various biomolecules such as antibodies, providing high binding capacity and sensitivity[72]. In diagnostic applications, Au NPs conjugated antibodies have gained much attention lately due to their rapid detection of biomolecules

by the appearance of a band on a test strip and a simultaneous increase in sensitivity. The integration of Au nanoparticles in the LFA kit, makes it very reliable for detection and confers several benefits: 1. acting as an effective substrate for binding of various antibodies 2. Improving absorption and detection limit, due to their exceptional optical properties, 3. providing a direct means for visualization of the result, which is accompanied by a persistent red color[73]. Despite the numerous advantages of Au NPs, their applications can be limited due to low signal intensity, which directly resulting in insufficient sensitivity. A common approach to amplify the signal is to accumulate more Au nanoparticles at the test line. To this end, Shen et al. attached polyamidoamine dendrimers (PAMAM), a multifunctional, highly branched, water-soluble macromolecule, on the surface of the Au nanoparticles through electrostatic interactions to promote aggregation[74]. The research was based on the idea that the signal generated by the aggregation of smaller gold nanoparticles can be stronger than the signal generated by a single larger gold nanoparticle. In another study, to improve the sensitivity of LFA kits, Tran et al. employed silver enhancement method[75]. Using this strategy, the detection limit was increased by about 10-fold for detecting Staphylococcus aureus α-hemolysin. In this process, Au NP serves as a catalytic surface for reducing silver ions to their metallic forms with the help of an effective reducing agent. The deposition of silver on the Au NPs, which acts as a nucleation site, causes Au NP enlargement. Ultimately, the increased size leads to significant signal amplification in both the test and control lines[76].

Quantum Dots

Quantum dots (QDs) are semiconductor nanoparticles with high quantum efficiency and narrow emission bandwidth, and can emit significant fluorescence upon exposure to UV light. The bright luminescence and stability of this class of nanoparticles make them suitable for use in highly sensitive biosensors for quantitative detection of both infectious and chronic diseases[77]. By changing the particle size, the color of the light emitted from the quantum dots also changes, enabling multiple analyses. In clinical diagnostics, the importance of multiple detection is increasingly recognized, as there is frequently a critical need to identify multiple biomarkers from the same samples to provide more definitive information. To increase the coupling efficiency of quantum dots, EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) and NHS (N-Hydroxysuccinimide), which are carboxyl-reactive cross-linkers, are used for labeling. EDC facilitates aqueous cross-linking, and DCC (DCC) is used for non-aqueous methods. The -COOH group of quantum dots is activated by these cross-linkers, allowing attachment to amines via amide bonds, thereby improving the specificity and sensitivity of LFA in detecting the target analyte. Quantum dots (QDs) are functionalized with various types of ligands to enhance stability, solubility, and binding to biological molecules such as DNA, enzymes and proteins[78]. Extensive research has been conducted on the use of quantum dots in LFA systems. In a study by Bock et al, silica-coated CdSe@ZnS quantum dots were fabricated through reverse microemulsion to detect prostate-specific antigen (PSA)[79]. Anti-PSA antibodies were subsequently attached to the surface of the silica-coated CdSe@ZnS nanoparticles. This LFA kit detected PSA with LOD of 1.0754 ng/mL, demonstrating sufficient sensitivity for detection of prostate cancer. In an interesting study for the simultaneous quantitative detection of tumor markers CEA (carcinoembryonic antigen) and AFP (alpha-fetoprotein) based on quantum dots (QD), a diagnostic kit was developed that used CdSe/ZnS quantum dots with wavelengths of 620 nm and 546 nm for the corresponding antibodies[80]. The test was designed to include one line for both markers and one control line. The LOD for AFP was 3 ng/mL and for CEA, 2 ng/mL, which were confirmed by high sensitivity (93% for AFP, 87% for CEA) and high specificity (94% for AFP, 97% for CEA) using 130 clinical samples. Despite the many advantages of quantum dots as labels, their observed toxicity often prevents their practical application, and in this regard, many studies have been carried out to reduce the toxicity of these nanoparticles. Eco-friendly Cu:Zn−In−S/ZnS quantum dots were synthesized by Wang et al. for the detection of tetanus antibodies[81]. The LOD achieved was 0.001 IU/mL, which was much lower than the gold nanoparticle-based LFA. In 2025, a new method for simultaneous detection of two biomarkers, TNF-α (tumor necrosis factor-α) and BDNF (brain-derived neurotrophic factor), was developed by Wu et al. using quantum nanobeads and succeeded in achieving low detection limits (3.39 pg/mL for TNF-α and 4.13 pg/mL for BDNF)

[82]. In this research, quantum dots were coated on the surface of the SiO2 core, enhancing the fluorescence intensity of the quantum nanobeads, and enabling efficient and precise bioanalysis. In addition to the aforementioned advantages, the proposed technique also features a smartphone-based readout, which could be considered a promising and economical option for point-of-care glaucoma screening.

Upconversion Nanoparticles

Upconversion nanoparticles (UCNPs) are regarded as lanthanide-doped particles that have garnered remarkable attention in diagnostic and therapeutic applications due to their distinctive ability to alter near-infrared (NIR) light (wavelengths >700 nm) into higher-energy visible (400–700 nm) or ultraviolet light (UV, <400 nm), a process known as upconversion[83]. This process was initially identified in the mid-1960s[84], relies on nonlinear optical phenomena, in which some of photons are sequentially absorbed through long-lived intermediate energy states, causing anti-Stokes emission-light emitted at a shorter wavelength than the input light[85]. UCNPs exhibit similar optical properties to QDs, including high optical stability, narrow emission bandwidth, adjustable emission and long lifetime, however unlike QDs that absorb UV light, potentially harmful to biological samples, these particles absorb near-infrared (NIR) light that does not disrupt biological samples. In 2024, a novel approach was presented by Chen et al. for multi-mode lateral flow assay (LFA) based on UCNPs to identify microRNAs, capable of generating three distinct signals on a strip. The Au-DTNB@Ag NPs featuring a core-shell structure, quench the fluorescence of UCNPs via the Förster resonance energy transfer (FRET) mechanism. The system detects microRNA-21 within a range from 2 nM to 1 FM, with similar results obtained from signal amplification methods. This biosensor identified patients with lung cancer and periodontitis, showing potential for disease prediction and diagnosis[86]. Infectious bacterial diseases result in millions of human deaths every year and also threaten water and food safety, making the development of rapid and reliable diagnostic methods extremely urgent. For this purpose, a luminescence sensor was developed by Arrai et al. using NaYF4 UCNPs. The synthesized UCNPs then doped with Tm3+ or Er3+ and COOH-PEG4-COOH was used for coating. The surface functionalization was done with either polymyxin-B or vancomycin, enabling it for selective targeting of Gram-positive and Gram-negative bacteria. The gold nanoparticles in the structure quench the UCNPs emission upon binding to bacteria. The sensor operates effectively in the range of 0.05 to 5 × 105 CFU/mL and demonstrates strong correlation with actual number of bacteria, making it useful for detection in various environments[87].

Recent Advancements in Optical Nanoparticle-Based LFIA for Bacterial Detection

Enhanced Sensitivity through Advanced Optical Nanomaterials

Recently, a novel method for bacterial identification was developed by combining LAMP and a gold nanoparticle-based lateral flow biosensor (LAMP-LFB), which is capable of rapidly, sensitively, and specifically detecting different Chlamydia trachomatis species. The entire test process (including DNA extraction, LAMP reaction, and final reading) is completed in less than 60 minutes, and its detection limit reaches 50 copies/mL. This test, with 100% accuracy and specificity, showed no cross-reactions with other pathogens. In addition to being fast and easy to perform, LAMP-LFB does not require special equipment, and the results can be interpreted visually. This technique is considered a very suitable option for point-of-care (POC) and rapid screening, especially in underserved and resource-poor areas. The mechanism of the gold nanoparticle-based LAMP-LFB assay for rapid detection of Chlamydia trachomatis involves three main steps: The sample is first extracted for genomic DNA, and a loop-linked isothermal amplification (LAMP) reaction is conducted using a primer set specific for the ompA gene. The two main primers are fluorescent and biotin-labeled. Afterwards, the LAMP products are simultaneously labeled with FAM and biotin and transferred to the lateral flow sensor (LFB). A capillary action is employed in this step to move the products along the LFB strip, in which they are bound in the test region by anti-FAM antibody and in the control region by biotin-BSA and gold nanoparticles (GNP). The presence of two red lines, one in the test region (TL) and the other in the control region (CL), indicates a positive sample; if only CL appears, the sample is considered negative [88].

An interesting method for detecting bacteria was developed by Morales-Narváez et al. In this study, quantum dot nanocrystals (CdSe@

ZnS, streptavidin-QDs) and graphene oxide (GO) were used as experiment and control lines on a nitrocellulose membrane to produce a photoluminescent lateral immunoassay (LFA). For fabrication, QDs are conjugated with anti-E. coli antibody and the test line with these Ab–QDs (concentration ~1.5 nM QDs and 100 $\mu g \cdot mL^{-1}$ Ab) and the control line with QDs without antibody are placed on nitrocellulose with an Isoflo dispenser, and an aqueous GO dispersion is used for detection at 90 $\mu g \cdot mL^{-1}$. The mechanism of action is based on the photoluminescence decay of QDs itself by resonance energy transfer (quenching) to GO: when the analyte (bacteria) is not present, the distance between QD (donor) and GO (acceptor) is very small and the fluorescence is "off". However, if bacteria bind to Ab–QDs in the test line, the size and physical distance between donor and acceptor increase (beyond the effective FRET range ≈ >20 nm) and quenching inhibition does not occur (the test line remains "on"), so the QTL/QCL ratio is used for detection. The reader uses a 365 nm excitation source and a ≈670 nm emission filter, and optimal conditions (QD ≈1.5 nM, GO ≈90 $\mu g \cdot mL^{-1}$) have been determined. The reported detection limit is ~10 $CFU \cdot mL^{-1}$ in standard buffer and ≈100 $CFU \cdot mL^{-1}$ in mineral water and milk samples [89].

Another study, conducted in 2023, examined the use of gold cauliflower nanoparticles (Au nanoflowers) modified with p-mercaptophenylboronic acid (PMBA) to develop an antibody-independent lateral immunoassay (MCI-LFIA) for rapid and accurate detection of bacterial urinary tract infections. In this system, AuNF-PMBA nanoparticles were used as multimodal markers with color, Raman, and photothermal signals due to their high ability to covalently bind to diol structures present in the bacterial wall. The mechanism of action is based on the direct binding of AuNF-PMBA to bacteria without the need for antibodies, which besides reducing the cost and preparation time, also significantly increases detection sensitivity. The structure of gold cauliflower nanoparticles with a size of about 100 nm was achieved through the growth method of 60 nm initial gold grains, and surface modification with PMBA helps to increase the ability to adsorb bacteria. This system has a limit of detection (LOD) of 103 colonies per milliliter (cfu/mL) for E. coli detection visually, for Raman and photothermal signals, the detection limit has been reduced to 102 cfu/mL, a three-order improvement over traditional antibody-based approaches [90].

Another study developed a novel label-free strip sensor for the detection of Salmonella enteritidis that uses nitrogen-rich carbon nanoparticles with a positively charged surface (pNC). In order to synthesize these nanoparticles, it is necessary to use a calcination and etching reaction method, in which nitrogen-containing organic precursors are carbonized at high temperatures, followed by surface modification with nitric acid to obtain a stable positive charge. The sensor's mechanism of action is based on the formation of a nanoparticle-bacteria complex through electrostatic interactions and hydrogen bonding between pNC and the bacterial cell wall. After the formation of this complex, it is specifically captured by the antibacterial monoclonal antibody (McAb) located on the test strip line, and the aggregation of nanoparticles causes a color change in the test line, which can be observed with the naked eye. This system has a very low detection limit of 102 CFU/mL and a wide linear range from 102 to 108 CFU/mL, which allows for rapid and sensitive detection of Salmonella enteritidis in a variety of food samples. The main advantages of this sensor include eliminating the need for labeling nanoparticles and using only one antibody to increase speed, reduce costs, improve ease of use, and maintain high sensitivity. It also demonstrates the applicability to complex food samples with recovery efficiencies between 85 and 110%, demonstrating the system's resistance to the effects of different matrices [91].

Multiplexing Strategies for Simultaneous Bacterial Identification

Recently, researchers developed a portable diagnostic system that simultaneously detects two pathogenic bacteria (P. carotovorum subsp. brasiliense and E. coli O157:H7) in fresh agricultural products using two powerful techniques. In this method, multiplex PCR is first used to simultaneously amplify genes specific to each bacterium. The PCR products are then transferred onto a dual lateral flow assay (LFA) strip, which has two separate detection zones, thanks to its labelling features. This design results in a separate visual signal for each bacterium on a single strip, so that each test line represents the presence of a specific bacterium species. This system has various advantages, such as portability

(with a portable PCR device), high sensitivity, speed (the whole process takes approximately 60 minutes), and reduced laboratory equipment usage. The detection limit of this multiplex PCR system with dual lateral flow strip for both studied bacteria is reported to be between 10 to 100 colony-forming units per milliliter (CFU/mL). This design mechanism enables rapid, accurate, and multi-bacterial identification for food safety and quality control [92, 93].

In a study published in 2021, which demonstrated a very low and suitable limit of detection for the simultaneous detection of two different bacteria, an advanced lateral flow strip-based fluorescence immunoassay method was introduced that uses silica quantum dots (Si@QD) as a fluorescent marker for the simultaneous detection of two food-borne pathogens, E. coli O157:H7 and Salmonella typhimurium. The double quantum dot shell of these nanoparticles enhances their fluorescence intensity, stability, and uniform dispersion. The mechanism of action is that fluorescent nanoparticles are impregnated with the sample and placed directly on the LFA strip, and a dual-channel fluorescence signal is generated from two separate test lines for each bacterial species, allowing rapid quantitative and qualitative detection in less than 15 minutes. Compared to conventional gold nanoparticle-based methods, this system can detect 50 cells per milliliter, which is approximately 200 times more sensitive. Besides their high sensitivity, this method has features such as excellent stability, high specificity, and ease of test execution, and is considered a powerful and suitable tool for monitoring food safety in field trials and real-world application environments. Across numerous industries, this novel approach improves the detection of food pathogens, leading to greater accuracy, speed, and reliability [94].

In an innovative study by Zhi and colleagues in 2025, a multi-mode lateral flow (LFIA) sensor was designed for the rapid detection of Klebsiella pneumoniae. In this research, multi-metallic Au@Au@Ag/Pt nanoenzyme was utilized with a core–gap–shell structure. These nanoparticles were fabricated by the simultaneous reduction of $AgNO_3$ and H_2PtCl_6 on the Au@Au core. 4-MBA molecule was also placed as a spacer and Raman reporter in the gap to create electromagnetic "hot spots" to enhance the SERS signal. After conjugation with specific antibodies, these nanoprobes were used in the LFIA strip for three types of signal readout:

CM-LFIA: Colorimetric detection with a 104 CFU/mL limit of detection.

CL-LFIA: Enhancing signal with Pt peroxidase-like activity (LOD=103 CFU/mL).

SERS-LFIA: high sensitivity readout using Raman signals (LOD = 38 CFU/mL).

The method demonstrates exceptional sensitivity, being approximately 200 times more effective than conventional nanogold-based lateral flow assays. It delivers results in under 12 minutes. By integrating enzymatic and plasmonic functions within a precisely engineered nanostructure, the approach provides a fast, accurate, and adaptable system for detecting nosocomial K. pneumoniae infections [95, 96].

Researchers in a novel study investigated the collaboration of antibodies and aptamers as an innovative mechanism for designing a Pseudomonas aeruginosa assay system on the LFIA platform. Specific aptamers were identified by the SELEX process. Then, AuNP-aptamer nonconjugate structures were prepared and the assembly of the test strip, including sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad, was performed. In the test line (T), the anti-P. aeruginosa antibody is immobilized, and in the control line (C), the anti-mouse IgG antibody is placed. The AuNP-aptamer nanoconjugates are mixed in solution with the sample and then applied to the strip. In the analyte's presence, the aptamers bound to AuNP bind to the bacterial surface and form the AuNP-aptamer-bacterium complex. This complex is captured by the immobilized antibody in the test region (T), and a red band appears. The detection limit was 2.34×10^2 CFU/mL, which competes with antibody-based systems [97, 98].

Integration with Portable Readers and Digital Quantification

A portable readout with digital measurement capabilities, if designed in a measuring device for a specific analyte, can naturally be used as a target base for the measurement of other analytes. One of the most important limitations of LFA systems is that, due to the nature of the system, quantitative signals cannot be measured. In many monitoring applications in the food safety and medical fields, a qualitative yes/no answer is not sufficient. The difference between a safe and a dangerous level depends on the amount of analyte detected [99].

For the LFA measurement system to be transformed into a quantitative and reliable one, two main factors must be met: 1. A readable device with a pre-installed calibration curve, 2. Compliance with the World Health Organization criteria for outpatient tests called REASSURED, which include portability and small size, a straightforward interface, battery operation with no power outlet, the ability to store output data, and signal specificity [100, 101].

In recent years, a hybrid system for the detection and quantification of food-borne bacteria E. coli O157:H7 was designed, which used a commercial LFA strip and a smartphone-based imaging module to create a portable, low-cost, and quantitative system. The function mechanism is that the LFA strip operates based on the antigen-antibody reaction in the presence of gold nanoparticles to detect the analyte. In the analyte's presence, a colored band is formed on the test line. The structure of this system comprises three key components: the cartridge containing the LFA, the Optical Imaging Box, and the Smartphone Cradle.

The working mechanism is that the LFA strip operates based on the antigen-antibody reaction in the presence of gold nanoparticles to detect the analyte. In the presence of the analyte, a colored band is formed on the test line. In this work, a 3D-printed imaging module consisting of an Optical Imaging Box (dimensions 2.5 × 3.8 × 2 cm) with a reflector, diffuser, and a plano-convex lens (≈×3 magnification) was designed as an interface between the flash/camera of the phone and the LFA strip to provide uniform exposure and a constant working distance. The RGB images from the crop strip area are converted to gray and averaged along the longitudinal coordinate. The background, because of nitrocellulose reflection, is modeled and removed with an eighth-order polynomial, and the resulting peak area/intensity is used to construct a calibration curve (up to 10^6 CFU/mL) and estimate bacterial concentration. In this study, the observed detection limit without enrichment was reported to be 10^4 CFU/mL for the Bioassay kit and 10^5 CFU/mL for the Rapid check. The mobile app also allows users to construct a calibration curve, report concentrations, save images, and geotag results [102].

In recent years, a study by Rajendran and his colleagues has used a combined approach based on fluorescence and smartphones for rapid detection of bacteria in a lateral flow assay system. In this unique device, silica nanoparticles doped with FITC and Ru(bpy) dyes were used as bright and stable probes. In FITC-SiNP, the dye is covalently attached to the silica network to prevent dye leakage, and the Ru(bpy)-SiNP micro-emulsion environment allows for efficient encapsulation of hydrophilic dyes. At this stage, each nanoparticle carries a large amount of dye molecules, indicating a strong signal.

After surface modification and covalent attachment to antibodies, these nanoparticles allow the accumulation of a large number of fluorophores on the surface of a bacterium, resulting in a significant increase in sensor sensitivity. After contacting the sample, the LFA strip is placed inside a lightweight optical module (with LEDs, pass/block filters, and a lens), and the fluorescent signal is recorded by the phone camera. Fluorescence intensity analysis is performed with a mobile application, and a detection limit of 10^5 CFU/mL has been achieved with no pre-enrichment. This technology combines the advantages of fluorescence with the portability of a smartphone and is considered a practical platform for rapid, field-based bacterial detection at minimal cost [103]. The following table summarizes the most significant and up-to-date studies (Table 2).

Challenges and Limitations of Optical Nanoparticle-Based LFIA Technologies

Lateral flow immunoassay (LFIAs) tests employing optical nanoparticles have revolutionized pathogen detection at the point of care owing to their simplicity, rapidity, and ease of use. Nonetheless, certain challenges and constraints continue to impede their broader application in clinical environments and the attainment of ideal outcomes.

Sensitivity and Limit of Detection (LOD)

Optical nanoparticles, including gold nanoparticles and quantum dots, are highly effective for signal detection owing to their distinctive light absorption and scattering characteristics. Nonetheless, their sensitivity continues to be restricted. Larger nanoparticles scatter light better, but their diffusion is less efficient, which slows down the assay kinetics and limits the increase in sensitivity to a specific nanoparticle size threshold [114, 115]. Furthermore, conventional fluorescent labels do not perform very well, which limits their sensitivity.

Efforts to improve fluorescence through metal-enhanced fluorescence (MEF) have shown some potential, but they introduce complexity and potentially reproducibility issues [116].

Sample Matrix Interferences and Specificity

Biological samples used in LFIA, such as blood, serum, and urine, present complex matrices

Table 2. Notable research on bacterial assay tech employing LFA and portable readers.

Readout system	Mechanism	Signal	Limit of detection	Target bacterium	Reference
Smartphone camera and color-saturation image analysis with a phone appliaction	Conventional LFAs with antibody-label (gold NP) and smartphone color-saturation quantification (color saturation metric to convert band intensity: concentration).	Colorimetric	2.40 CFUs ml−1	*Klebsiella pneumoniae*	[104]
Automated rotating 3D-printed device and smartphone imaging + app	Multi-step LFIAs: sequential reagent pads (sample → antibody-reagent pad → wash → chromogenic developer (DAB)), servo motor rotates pads automatically; smartphone images final strip for quantitative investigation.	Colorimetric	Visual reading ~5×104 CFU/mL; statistical LOD reported ≈ 100 CFU/mL under ideal conditions	*Escherichia coli* O157:H7	[105]
Smartphone and imaging cradle	Conventional LFIAs strips imaged in a controlled cradle; image features (RGB/L*a*b with SVM and KNN classifiers) extracted and classified to increase detection accuracy and lower subjective error.	Colorimetric	5 × 104 CFU/mL	*Salmonella spp.*	[106]
Smartphone imaging (fluorescent readout)	Fluorescent LFIAs using dual recognition (capture by IgG and antibiotic-based recognition), fluorescent labels read by smartphone with a simple optical module and filter set.	Fluorescent (image fluorescence intensity: quantification	102 CFU mL−1	*Staphylococcus aureus*	[107]
Integrated LFIAs + screen-printed electrodes (DEP trapping) and, portable readout (electrochemical/optical)	LFIAs combined with on screen printed electrodes that use dielectrophoresis (DEP) to trap/concentrate bacteria in the detection zone	Electrochemical/ colorimetric	15 CFU ml−1	*Salmonella*	[108]
Portable 3D-printed pretreatment device and graded LFA, and smartphone imaging	3D-printed pretreatment (sample cleanup / concentration) upstream of graded LFIAs to improve sample quality and detection performance; read by smartphone imaging.	Colorimetric (graded LFAs)	102 CFU/mL	*Staphylococcus aureus*	[109]
LFIAs and nanocomposite labels enabling dual readout (optical + another modality)	Multi-functional nanocomposite (magnetic/optical/other functionalities) integrated into LFIAs for label-free capture and dual readout (e.g., magnetic and or optical).	Magnetic-colorimetric/optica l	10 CFU mL−1	*E. coli* O157:H7	[110]
LFIAs and smartphone imaging for readout	Antibody cocktail immobilized on test line to broaden subtype coverage; smartphone quantification of line intensity for semi-quantitative readout.	Colorimetric	With LBs-LFIAs : 105 CFU mL−1 and with FBs-LFIA : 103 CFU mL−1	epidemic subtypes of group A *Streptococcus*	[111]
LFA with SERS readout (portable Raman /	3D membrane-like SERS nanostickers (Antibody-	bi-channel surface-enhanced Raman	9 cells mL−1	*Salmonella typhimurium*	[12]

Continued Table 2. Notable research on bacterial assay tech employing LFA and portable readers.

Readout system	Mechanism	Signal	Limit of detection	Target bacterium	Reference
smartphone-coupled spectrometer	labeled GO@Au/Ag nanostickers) enhance Raman scattering at test lines enabling multiplex SERS readout; bi-channel strip for parallel assays.	scattering: multiplex spectroscopic signal		(S. typhi), *Escherichia coli* (E. coli), *Staphylococcus aureus* (S. aureus), and *Listeria monocytogenes*	
Smartphone imaging app	$Cu_{2-x}Se$ nanocrystals used as readout tag enabling capture-antibody-independent signal generation (novel tag that interacts with bacteria or reporter chemistry).	Colorimetric/ photothermal/ tag-specific optical signal	2.65 × 105 CFU mL−1	*Escherichia coli* O157:H7	[112]
Smartphone imaging and photothermal readout (thermal camera or IR sensor)	TA-$TaTe_2$@lysozyme nanocomposite used as label enabling both colorimetric change and photothermal signal—integrated with smartphone for dual-mode detection.	Dual mode: colorimetric/ photothermal	93 CFU mL−1	*S. aureus*	[113]

that can interfere with nanoparticle behavior and antibody-antigen interactions. Non-specific adsorption of proteins and other biomolecules onto nanoparticles or membrane surfaces causes background noise and false signals, diminishing assay specificity and reliability. In particular, carbon-based nanoparticles—though showing highly sensitive detection—suffer from hydrophobicity and bio-conjugation challenges limiting their bio-applicability [116, 117].

Reproducibility and Stability

When nanoparticles are synthesized and functionalized, they often show differences from batch to batch, which can affect their size, shape, and surface chemistry. These variances influence the adherence of the components and their visual representation, leading to variable analytical results and complicating quality assurance.

Furthermore, nanoparticles such as SERS (surface-enhanced Raman scattering) labels and MEF-based particles have stability issues during storage and use, limiting their practical application [99, 115, 118].

Signal quantification and multiplexing

Most LFIA tests depend on colorimetric alterations visible to the naked eye, thereby limiting both sensitivity and quantitative accuracy.

Although optical detection devices can improve quantification, their cost and complexity reduce the accessibility and ease of use of LFIA tests at the point of care. Furthermore, multiplex detection is constrained by the overlap of optical signals and the challenges involved in concurrently managing multiple nanoparticle sensors without interference [119, 120].

Cost and Practical Implementation

The integration of advanced nanoparticles or signal amplification strategies often increases the cost and complexity of producing LFIA devices, thus limiting their accessibility for widespread use in resource-limited settings. Moreover, adding design components aimed at minimizing light reflection or improving readability can increase manufacturing costs by approximately 10–15% [114].

Although LFIA tests based on optical nanoparticles offer the potential for rapid bacterial detection at the point of care, further studies are needed to improve sensitivity, specificity, repeatability, quantification, and cost-effectiveness. Future developments will most likely include improved nanoparticle design, integration with portable optical detection devices, multifunctional nanoparticle platforms, and enhanced sample preparation techniques to reduce matrix effects and non-specific interactions.

Future Perspectives and Opportunities for Advancing LFIA in Bacterial Diagnostics

The development of lateral flow immunoassay (LFIA) tests in the field of bacterial diagnostics depends on improving sensitivity, enabling multiplex

detection, enhancing portability, and integrating digital technologies to meet clinical requirements and point-of-care testing needs.

Improved Sensitivity and Signal Amplification

Novel nanomaterials, such as upconversion nanoparticles, plasmonic nanoparticles, and metal-enhanced fluorescence probes, have significantly advanced detection capabilities. These compounds enhance signals and reduce detection thresholds, facilitating the identification of bacteria at minimal quantities. This is essential for the initial phases of infection [121, 122].

Multiplex Detection Capabilities

Future LFIA platforms are under development to facilitate the simultaneous detection of multiple bacterial pathogens or antibiotic resistance markers. The use of multi-labeled optical nanoparticles and spatial structuring on the test strips allows for the simultaneous analysis of several samples. This reduces the duration and sample volume required for diagnosis while improving clinical decision-making [121-123].

Smartphones and Portable Readers

The use of smartphone-based imaging and analysis applications for reading LFIA tests allows for the conversion of qualitative visual results into quantitative data. Recent developments in portable readers that employ LEDs, cameras, and machine learning enhance accuracy, usability, and accessibility, which are essential for decentralized healthcare in resource-constrained environments [16, 99, 124].

Combining Microfluidics with Sample Preparation

The integration of microfluidics with sample preparation enhances test outcomes by reducing interference from complex biological matrices and enabling automated operations in LFIA testing. The integration has made LFIA testing easier to employ in everyday clinical settings, while also making it easier to find and lessening the need for manual processes [16, 99].

Expansion to Antibiotic Resistance and Virulence Factor Detection

Next-generation LFIA assays are designed to identify not only bacterial presence but also antibiotic resistance genes and virulence factors, thereby supporting more precise treatment strategies. This expansion facilitates antimicrobial stewardship and addresses resistant infections [121, 123].

In conclusion, forthcoming developments in lateral flow immunoassay (LFIA) testing will focus on enhancing sensitivity via cutting-edge nanotechnologies, multiplexing, digital and portable detection, and integrated sample processing, thereby enabling rapid, cost-effective, and precise diagnosis of bacterial infections at the point of care.

CONCLUSION

Recent progress in nanoparticle-driven lateral flow immunoassays (LFIAs) has greatly boosted the effectiveness of point-of-care (POC) bacterial identification. As outlined in this overview the incorporation of optical nanomaterials, such as colloidal gold nanoparticles, quantum dots and up-conversion nanoparticles has significantly enhanced the analytical characteristics of LFIAs regarding sensitivity, specificity and detection limits. These advancements emphasize the expanding capability of LFIAs as mobile, affordable and easy-to-use diagnostic instruments, for both clinical and field settings.

Despite these accomplishments obstacles persist. LFIAs performance continues to be influenced by sample- variability, matrix interferences, limited signal production at very low analyte concentrations and the requirement for labor-intensive sample preparation in complex matrices. To address these constraints promising approaches—such as pre-concentration, silver enhancement, catalytic amplification, aptamer-based recognition elements and hybrid SERS-coupled platforms—are being actively developed and have the potential to convert LFIAs into ultra-sensitive and highly reliable diagnostic tools.

Anticipated advancements will likely concentrate on LFIA systems, automated sample-to-answer processes, minimized sample preparation and comprehensive integration, with smartphone detection and digital measurement. The development of next-generation nanostructures exhibiting optical properties, combined with portable analyzers and AI-driven analysis will enhance the accuracy and medical significance of LFIA tools. Overall, continued innovations in nanotechnology and optical signal amplification will position nanoparticle-based LFIAs as indispensable tools for rapid, accurate, and decentralized bacterial detection—particularly in low-resource settings and during infectious disease outbreaks.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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