

Review

Gold Nanoparticles-Based Colorimetric Assays Using Aptamers for Rapid Detection of Food- and Water-Borne Infections in Low-Income Countries

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Academic Editor: Paschalis Alexandridis

Special Issue: [Synthesis, Properties and Applications of Nanocomposite Materials](#)

Recent Progress in Materials
2026, volume 8, issue 3
doi:10.21926/rpm.2603005

Received: January 20, 2026
Accepted: July 06, 2026
Published: July 16, 2026

Abstract

Food and water are essential resources for human survival; however, the increasing global population, urbanization, and climate change have placed a significant strain on their availability and safety, with major implications for public health and economic stability. Microbial contamination of food and water remains a critical global challenge, with bacteria



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responsible for more than 90% of foodborne illnesses. In the past decade, pathogens such as *Salmonella*, *Campylobacter*, and *Listeria* have been the leading causes of outbreaks. In contrast, diseases such as listeriosis and cholera constantly affect several African countries, with rapidly escalating infection rates. Early detection of pathogens in food and water is essential to prevent the spread of infections and reduce associated morbidity and mortality. Although current laboratory-based and on-site detection methods are highly sensitive and reliable, they are often limited by long assay times, labor-intensive procedures, and the need for specialized equipment and trained personnel. These limitations highlight the urgent need for rapid, cost-effective, and user-friendly diagnostic tools suitable for point-of-care (PoC) applications, particularly in resource-limited settings. While recent studies have explored nanomaterial-based detection strategies, existing reviews largely provide descriptive summaries and often lack a critical comparison of emerging biorecognition elements and their practical applicability in low-resource environments. This review provides a comprehensive and critical evaluation of aptamer-integrated gold nanoparticles (AuNPs)-based colorimetric assays, with a particular focus on lateral flow assays (LFAs) for the rapid detection of food- and water-borne pathogens. Key aspects, including assay performance, advantages and limitations of aptamer-based systems, and their potential for deployment in low-income countries (LICs) are discussed. By highlighting current advances, existing challenges, and future perspectives, this review aims to support the development of accessible and scalable diagnostic platforms for improving food and water safety.

Keywords

Aptamers; food-borne pathogens; water-borne pathogens; gold nanoparticles; lateral flow assay; rapid diagnostics

1. Introduction

Food- and water-borne infections represent a significant global threat and remain a major concern, particularly in rural areas where food safety measures and sanitation are often inadequate. Approximately 48 million people acquire foodborne infections each year, resulting in 128,000 hospitalizations and 3,000 deaths [1]. Water-borne infections are also prevalent; however, the true burden of water-borne illnesses remains unclear, with estimates ranging from 7.15 to 50 million illnesses per year in the United States alone, caused by recreational water exposure [2]. These statistics indicate that outbreaks resulting from the consumption of microbiologically contaminated food and water can occur in both developed and developing countries. The severity and spread of these diseases can be reduced through access to advanced techniques capable of identifying pathogens at PoC. Conventional methods for detecting bacteria in food and water are highly sensitive; however, they have several limitations. These methods are often time-consuming, laborious, and require specialized training. Collectively, these limitations highlight the need for simpler detection tools suitable for PoC testing (PoCT).

Despite the growing body of research on nanomaterial-based diagnostic systems, there remains a need for a comprehensive and critical evaluation of emerging detection strategies that are both

effective and applicable in resource-limited settings [3]. Existing studies often focus on LFA components, such as nanomaterials or biorecognition elements [4], without adequately addressing their integration into practical diagnostic platforms. These studies review LFA strategies for the detection of food- and water-borne pathogens, developed using various bioreceptors (antibodies, aptamers, bacteriophages, peptides) and nanomaterial labels (AuNPs, quantum dots (QDs), magnetic NPs) [5, 6]. Additionally, limited attention has been given to the specific challenges associated with implementing these technologies in LICs [4], where access to rapid, affordable, and user-friendly diagnostic tools is mostly needed. The use of QDs [7] and magnetic NPs [8] will be limited in PoC applications due to their requirement for specialized readers. Immunoassays based on antibodies or nanobodies are not only costly but will require cold storage, which is a main issue in high-temperature regions. While nanobodies show superior properties to antibodies, the concept needs to be clinically validated [9]. The current review provides a comprehensive and critical analysis of aptamer- and AuNPs-based LFAs for the rapid detection of food- and water pathogens. The review examines the underlying principle, performance characteristics, advantages, and limitations, with particular emphasis on their potential PoC applications in low-resource settings. By highlighting recent advances, existing challenges, and future perspectives, this work aims to support the development of accessible and scalable diagnostic solutions for improving food and water safety.

2. Food- and Water-Borne Infections

Food-borne pathogens have caused large epidemics in many countries, resulting in millions of sporadic illnesses and chronic health complications. Pathogens of bacterial, viral, fungal, and parasitic origin have all been associated with food- and water-borne infections [10]. In addition, animal waste, sewage discharge, and industrial effluents are common sources of water contamination that significantly contribute to human infections through the consumption of contaminated drinking water, as observed in LICs [11]. Bacterial contamination is the most common cause of food-borne infections [12] and is also frequently associated with water-borne diseases; several examples are highlighted in Table 1.

Table 1 Food- and water-borne pathogens and their symptoms.

Pathogen	Type	Disease	Symptoms	Refs
Enterohemorrhagic <i>Escherichia. coli</i> (<i>E. coli</i>) O157:H7	Water- and food-borne	Haemolytic uremic syndrome, thrombocytopenia, haemolytic anaemia	Vomiting, diarrhoea, abdominal discomfort, fever, haemorrhagic colitis, dehydration, shock	[13]
<i>Listeria monocytogenes</i>	Food-borne	Listeriosis	Febrile gastroenteritis, sepsis, meningitis, rhombencephalitis, perinatal infections, abortions	[14]
<i>Campylobacter</i>	Food-borne	Campylobacteriosis	Vomiting, fever, malaise, abdominal discomfort, bloody or watery diarrhoea	[15]
<i>Salmonella enterica</i> (<i>S. enterica</i>)	Water- and food-borne	Salmonellosis	Typhoid, diarrhoea, bacteraemia, chronic asymptomatic carriage	[16]
<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	Food-borne	Gastroenteritis	Severe sickness, cramping, vomiting, prostration skin soft tissue infections, pneumonia, food poisoning, toxic hock	[17]

Food- and water-borne epidemics caused by bacterial contamination have been observed throughout history. *L. monocytogenes*, *E. coli*, *Salmonella*, and *Campylobacter* are among the commonly reported pathogens responsible for outbreaks across the globe. A listeriosis outbreak associated with the consumption of hot dogs and processed deli meats was reported in 10 US states in 1998 [18]. Between 2017 and 2018, the largest and most severe outbreak in recorded history occurred in South Africa [19]. The outbreak was linked to ready-to-eat processed meat products produced by Tiger Brands Limited (South Africa). The economic impact of the outbreak was substantial, with losses estimated at approximately US \$260 million. In addition, hospitalization costs for patient recovery were estimated at US \$10.4 million, while productivity losses and export revenue reductions amounted to approximately US \$15 million [20].

Another notable outbreak involved *E. coli* O157:H7 infections linked to the consumption of undercooked ground beef at fast-food restaurant chains in the western United States. [21]. In 2015, a *Salmonella* outbreak emerged across 4 Canadian provinces and was associated with contaminated frozen chicken products. Similarly, a *S. Saintpaul* outbreak in South Australia in 2016 was linked to the consumption of raw mung bean sprouts [22]. A *S. Virchow* outbreak associated with powdered meal replacement products was reported across multiple US states in 2016. Additionally, an *S. Poona* outbreak affecting 40 US states was traced to cucumbers imported from Mexico. In South Africa, *S. typhimurium* was the leading source of salmonellosis until it was overtaken by *S. enteritidis* in 2011 [23]. *Campylobacter* outbreaks have also frequently been associated with water-borne infections in many countries. For example, a large outbreak of *Campylobacter* gastroenteritis

occurred in a Danish town in June 2009 following unusually heavy rainfall that contaminated the local water supply [24].

3. LFAs for Detection of Pathogens at a PoC

Food- and water-borne pathogens pose a serious risk to public health; it is therefore crucial to identify their sources and contain outbreaks promptly [25]. Conventional microbiological and biochemical tests for the detection of bacterial contaminants in the laboratory or on-site (Figure 1) can provide information about the number and type of microorganisms present. However, these tests are often constrained by long assay times and frequently require additional enrichment steps when pathogens are present in low concentrations [26]. Because culture- and filtration-based methods have prolonged turnaround times, more rapid detection techniques, such as immunoassays and polymerase chain reaction (PCR)-based methods, have been developed to improve pathogen detection [27, 28].

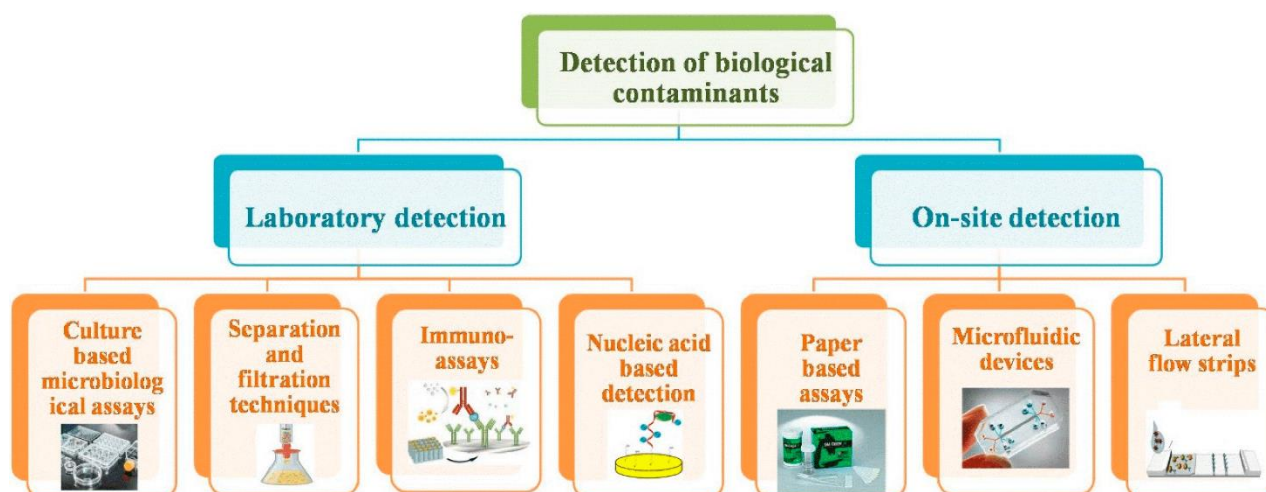


Figure 1 Techniques used to detect microbiological contaminants. Reprinted with permission from MDPI [29].

PoCT approaches have been explored for the on-site detection of various analytes, including those of microbial origin, to combat food- and water-borne outbreaks [30]. These approaches are based on fluorescence, electrochemical, or colorimetric detection techniques and may utilize platforms such as in-solution colorimetric assays, test strips, microchips, or portable meters. Among these methods, electrochemical and colorimetric PoCT systems offer several advantages, including faster detection times, reduced sample volume requirement, and user-friendly operation [31]. In addition, results from colorimetric assays such as LFAs can be easily interpreted visually [32]. LFAs satisfy the World Health Organization (WHO) ASSURED criteria for ideal rapid PoC systems in developing countries: A - Affordable, S - Sensitive, S - Specific, U - User-friendly, R - Robust and rapid, E - Equipment-free, and D - Deliverable to end users [33].

LFAs have advanced significantly over the years, leading to the commercialization of numerous diagnostic assays that have improved PoCT globally [34]. LFA-detectable analytes range from glucose [35], human chorionic gonadotropin (hCG) [34], to antibodies, antigens, nucleic acids, and bacterial pathogens [29]. Currently, several commercially available home-based tests are available for detecting various bioanalytes, including urine tests for hCG (the pregnancy hormone) and blood

tests for biomarkers associated with Human Immunodeficiency Virus-1, diabetes, cholesterol, and hepatitis C [36].

LFAs are compatible with a wide range of human biological samples, including whole blood, plasma, urine, stools, saliva, cerebrospinal fluid, and nasal swabs. In addition, these assays have been explored for the analysis of dietary matrices such as juices, cereals, meat, and veggies, as well as environmental samples including water and soil [37]. These capabilities highlight the potential of LFAs as a viable option for detecting microbial contaminants in both food and water. Importantly, PoCT platforms enable clinicians to make timely therapeutic decisions at PoC, often within minutes [38]. This rapid diagnostic capability is particularly important during outbreaks, where infections can be detected early to prevent further spread [39].

3.1 Aptamers and AuNPs Enhance the Performance of LFAs

The integration of aptamer technology with nanotechnology has led to significant advancements in improving LFAs performance. In these systems, aptamers can replace antibodies as biorecognition elements, while nanomaterials serve as detection labels [40]. The combination of these technologies enables the development of cost-effective LFAs that are particularly suitable for use in LICs, where healthcare systems often face challenges such as inadequate infrastructure and limited availability of skilled personnel [41]. Importantly, these systems do not require specialized storage conditions and can be performed and interpreted by end users [42]. Recent studies have demonstrated the versatility of aptamer-functionalized AuNPs in colorimetric biosensing platforms, highlighting their improved sensitivity and adaptability for detecting a wide range of targets [43]. Consequently, such strategies could be adopted for the quality control of food and water resources in order to reduce infections and prevent disease outbreaks. Figure 2 shows a consolidated overview of an aptamer-AuNPs-based LFA mechanism and its application in LICs for the detection of pathogens at a PoC.

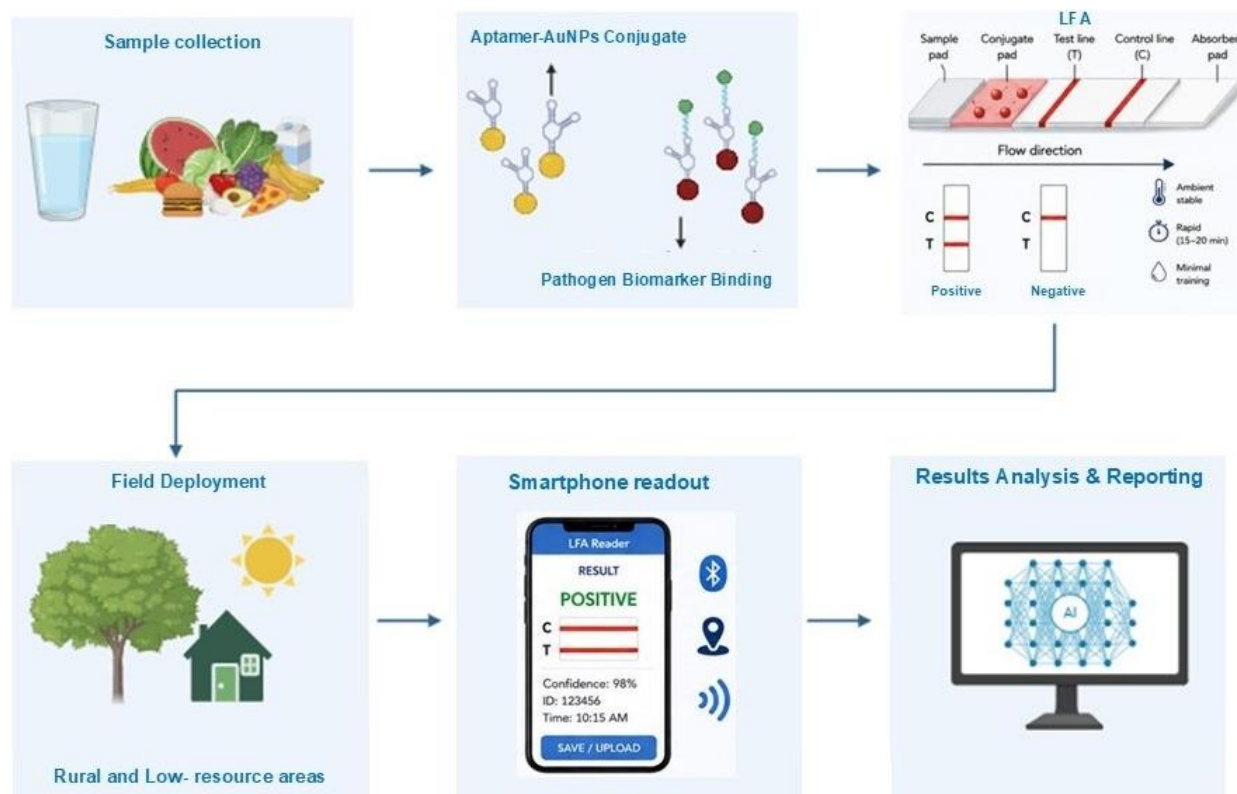


Figure 2 Schematic illustration of an aptamer-functionalized AuNP-based LFA for the detection of food- and water-borne pathogens. The workflow includes (i) sample introduction (food or water), (ii) interaction of target analytes with aptamer-conjugated AuNPs, (iii) capillary migration along the strip, and formation of visible test and control lines via specific binding interactions, and (iv) result interpretation, including potential integration with smartphone-based readout systems.

3.1.1 Aptamers as Biorecognition Elements in LFAs

Aptamers are single-stranded oligonucleotides that can fold into distinct three-dimensional (3-D) structures, including loops, hairpins, pseudoknots, branches, and quadruplexes [44]. These 3-D structures are essential for enabling aptamers to bind to their targets with high specificity and selectivity [45]. Unlike antibodies, aptamers can theoretically be produced against any desired target [46], including proteins, whole cells [47], toxins, non-immunogenic targets, or even small molecules [48]. Additional advantages of aptamers over antibodies include lower production costs, high stability, minimal batch-to-batch variation, extended shelf life [49], and ease of modification via various chemical strategies [50]. Due to these advantages, aptamers have been widely used in colorimetric assays, including LFAs [51], as an alternative to antibodies for detecting pathogenic microorganisms in food and water samples [52]. While antibody-based LFAs remain widely used due to their well-established performance and high affinity toward target antigens [53, 54], aptamers offer several practical advantages for PoC diagnostics. In contrast to antibodies, which require biological production systems and controlled storage conditions, aptamers are chemically synthesized, enabling scalable production with lower costs and improved stability under varying environmental conditions [40, 54, 55]. These characteristics make aptamers attractive for diagnostic applications in resource-limited settings [53, 55]. However, despite their promising attributes,

aptamer-based detection systems still face several challenges, including the complexity of the Systematic Evolution of Ligand by Exponential Enrichment (SELEX) selection process, potential cross-reactivity with structurally similar targets, and matrix interference in complex food and environmental samples [56, 57]. The SELEX process typically involves multiple iterative rounds of binding, separation, and amplification, which can be time-consuming and labor-intensive, often requiring several cycles to obtain high-affinity aptamers [58, 59]. In addition, amplification bias during PCR may result in the preferential enrichment of sequences with higher amplification efficiency rather than true binding affinity, thereby compromising the overall selection quality [58]. Nanomaterial-assisted SELEX strategies, such as graphene oxide-based platforms, have been explored to enhance target binding efficiency and improve the selection of high-affinity aptamers, particularly for small-molecule detection. However, it still follows the lengthy SELEX process [60]. Furthermore, the selection process can be influenced by experimental conditions, which may affect reproducibility and limit the robustness of aptamer performance across different studies [58, 59]. Although recent advances such as microfluidic SELEX and high-throughput screening approaches have improved selection efficiency and reduced processing time, challenges related to scalability and large-scale production of aptamers remain [61, 62]. In recent years, *in silico* selection of aptamers has proven to be more cost-effective and highly efficient in producing target-specific aptamers in a short period [60].

Overall, aptamer-based LFAs offer several advantages over conventional antibody-based systems. A summary of the key advantages and limitations of aptamer- and antibody-based LFAs is presented in Table 2. These include lower production costs, enhanced stability under varying environmental conditions, ease of chemical modification, and minimal batch-to-batch variation. These features make aptamers particularly attractive for PoC applications, especially in resource-limited settings. However, several limitations remain, including the complexity and time-intensive nature of the SELEX process, potential cross-reactivity with structurally similar targets, and reduced performance in complex sample matrices due to interference effects. In contrast, antibody-based systems benefit from well-established production protocols and consistently high binding affinity. Still, they are limited by higher production costs, batch-to-batch variability, and dependence on cold-chain storage. Therefore, while aptamers represent a promising alternative to antibodies, further optimization of selection processes and assay design is required to fully realize their potential in practical diagnostic applications.

Table 2 Comparative advantages and limitations of aptamer- and antibody-based LFAs.

Feature	Aptamer-Based LFAs	Antibody-Based LFAs	Refs
Production method	Chemical synthesis is highly reproducible and scalable	Biological production (animals or cell culture systems)	[40, 54, 55]
Production cost	Lower due to synthetic processes	Higher due to biological production requirements	[40, 54, 55]
Stability	High thermal and chemical stability, tolerant to varying environmental conditions	Sensitive to temperature and pH, requires controlled storage (cold chain)	[40, 49, 54, 55]
Batch variability	Minimal batch-to-batch variation	Possible variation between production batches	[49]
Target range	Broad can bind proteins, whole cells, toxins, small molecules, and non-immunogenic targets.	Limited to immunogenic targets	[46-48]
Ease of modification	Easily functionalized with chemical groups for assay design	Limited flexibility for chemical modification	[50]
Binding affinity and specificity	High specificity and affinity, but dependent on selection quality	Consistently high affinity and specificity	[45, 53, 54]
Selection/production challenges	The SELEX process is time-consuming, labor-intensive, and subject to PCR bias.	Well-established production protocols	[58, 59]
Matrix effects	Susceptible to interference in complex food and environmental samples	More robust in complex matrices	[40, 56, 57]
Field applicability	Highly suitable for PoC and resource-limited settings due to stability and cost-effectiveness.	Limited by storage requirements and higher costs	[53, 55]

3.1.2 AuNPs in the Development of LFA for Food- and Water-Borne Pathogens

LFA test results are typically translated into an optical signal that can be interpreted with the unaided eye. The visible signal is generated by colored detection probes conjugated to the biorecognition molecules (in this case, aptamers) after binding to immobilized targets at the test and control lines [63]. Examples of such probes include latex beads [64], and graphene oxide [40]. Detection probes must be detectable at low concentrations and retain their functional properties after modification with biorecognition molecules [65]. Traditional detection probes often exhibit limited sensitivity and may be susceptible to photobleaching. Therefore, nanomaterial-based labels have been introduced to overcome these limitations.

NPs are materials with at least one dimension in a size range between 1 and 100 nm. They can be broadly classified into organic and inorganic nanomaterials, resulting in a wide range of NPs such

as metallic, polymeric, lipid-based, and carbon-based NPs. Nanomaterials possess a large surface area-to-volume ratio, which allows efficient conjugation with various biomolecules for specific applications [48]. Among these nanomaterials, AuNPs, silver NPs, and QDs have been widely used as visual detection labels for qualitative assays [34]. However, some nanomaterials require specialized readout instruments. For example, QDs in fluorescence-based LFAs require an additional excitation device [7]. In contrast, AuNPs have gained significant popularity because they enable visual detection without additional equipment [66]. Moreover, AuNPs exhibit long-term stability and high biocompatibility [67]. The broader application of AuNPs in bioimaging and colorimetric assays as contrast agents, signaling probes, and fluorescence probes have been extensively reviewed elsewhere [4, 68], therefore, only their application as detection probes in LFAs will be discussed herein.

AuNPs are commonly used as chromogenic labels in colorimetric diagnostic systems, including LFAs and in-solution colorimetric assays [69, 70]. Due to their localized surface plasmon resonance (LSPR), AuNPs appear red in solution, which makes them particularly useful in the development of colorimetric detection systems that can be interpreted with the naked eye [71]. Their optical properties are influenced by their shape and size, both of which play important roles in determining the efficiency of LFAs [68]. Spherical AuNPs are preferred for LFAs because they exhibit good colloidal stability over a wide size range, stable optical properties, and reduced nonspecific interactions [72].

AuNPs-based LFAs have been widely applied for bacterial detection in biological, food, and environmental samples [73]. For example, Figure 3A illustrates an AuNPs-based LFA for the detection of whole *E. coli* O157:H7 in solution. In this study, AuNPs with different shapes (spherical, flowerlike, and popcornlike) were used as signal probes (Figure 3B), all of which produced detectable signals that were dependent on AuNP size and shape. These findings demonstrate that pathogens can be rapidly and cost-effectively detected using LFAs [74]. Most AuNPs-based colorimetric biosensors developed for the detection of pathogenic bacteria have focused on milk [75] and meat samples. However, other food matrices such as seafood [76], salads and fresh fruits [77], home-prepared foods [78], and water samples have also been investigated.

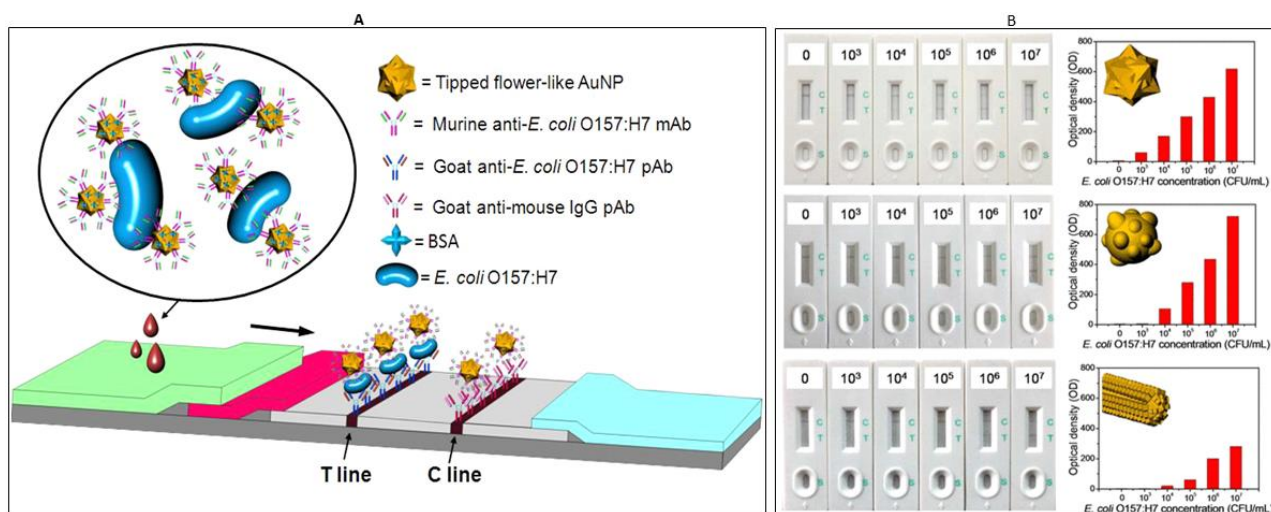


Figure 3 AuNP-based lateral flow immunoassay (LFIA) for the rapid detection of *E. coli* O157:H7 in solution. (A) Principle and (B) sensitivity of AuNP-based LFIA. Reprinted with permission from ACS [74].

Food contamination can occur not only on farms but also during processing stages, storage, and transport. During cold storage, psychotropic bacteria such as *Pseudomonas* and *Acinetobacter* spp. commonly grow in milk [79], while contamination with *S. aureus*, *E. coli*, and *Campylobacter* may occur if hygienic practices are not properly maintained [12]. Food products are highly susceptible to microbial contamination and must therefore be monitored at every stage of processing. Rapid colorimetric assays can be used on-site at different production stages to help prevent food-borne outbreaks [12]. Although many assays employ antibodies for their excellent target selectivity and affinity [80], antibodies also have several limitations. These include limited specificity between target antigens and homologous unrelated epitopes, high production costs, thermal and chemical instability, and the inability to target non-antigenic molecules [81].

Aptamers, on the other hand, exhibit low immunogenicity and can bind to their target molecules with high selectivity [82]. In addition, their thermal stability reduces the need for cold storage, which makes them particularly suitable for PoCT in LICs. The use of aptamers, either alone or in combination with antibodies in LFAs, has demonstrated improvements over conventional LFIAs, as reviewed elsewhere [53]. Figure 4A and Figure 4B illustrate that aptamers can be used in competitive and sandwich formats, respectively. An aptamer-AuNPs-based LFAs have been used for the detection of *S. typhimurium* [83, 84] as well as in multiplex system capable of simultaneously detecting *S. typhimurium*, *E. coli* O157:H7, and *S. aureus* in 30 food samples including milk, chicken, and frozen food products [85].

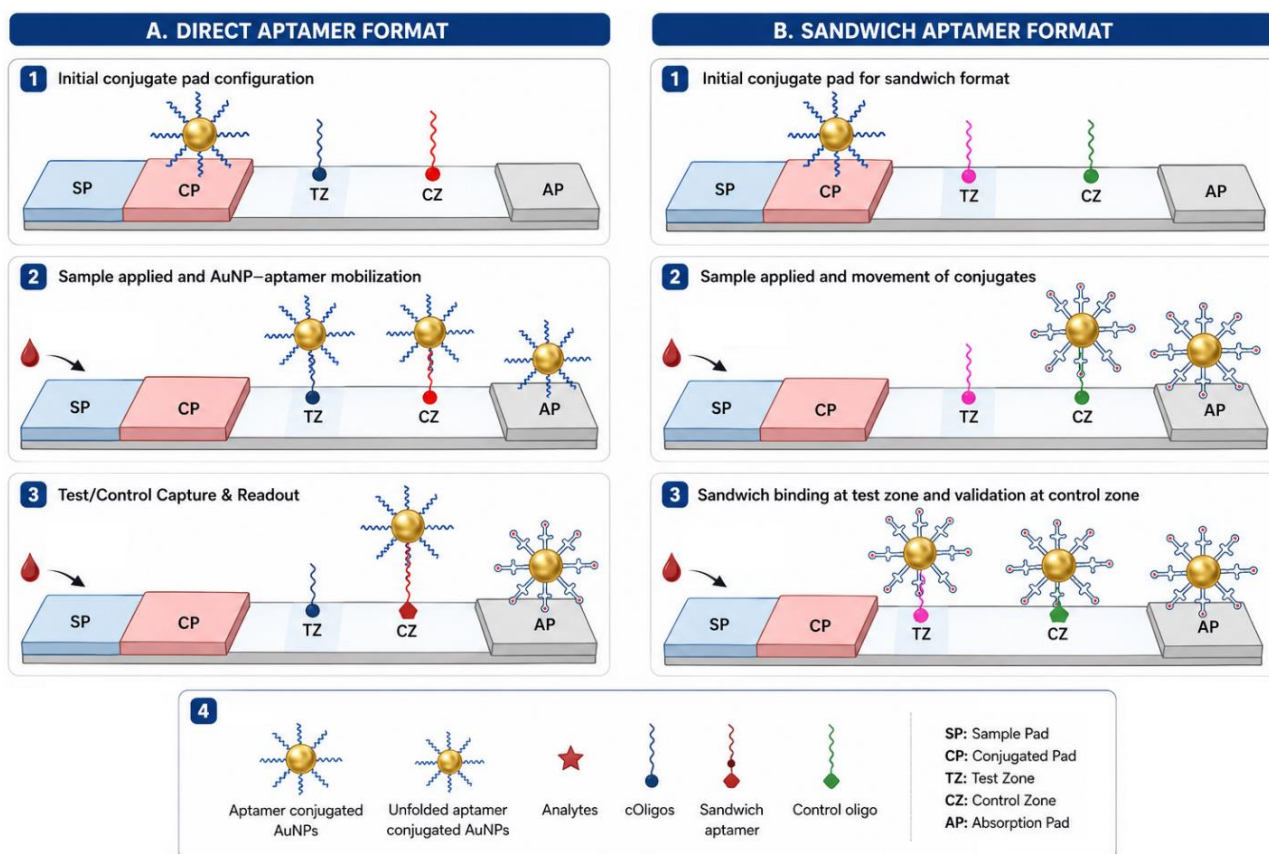


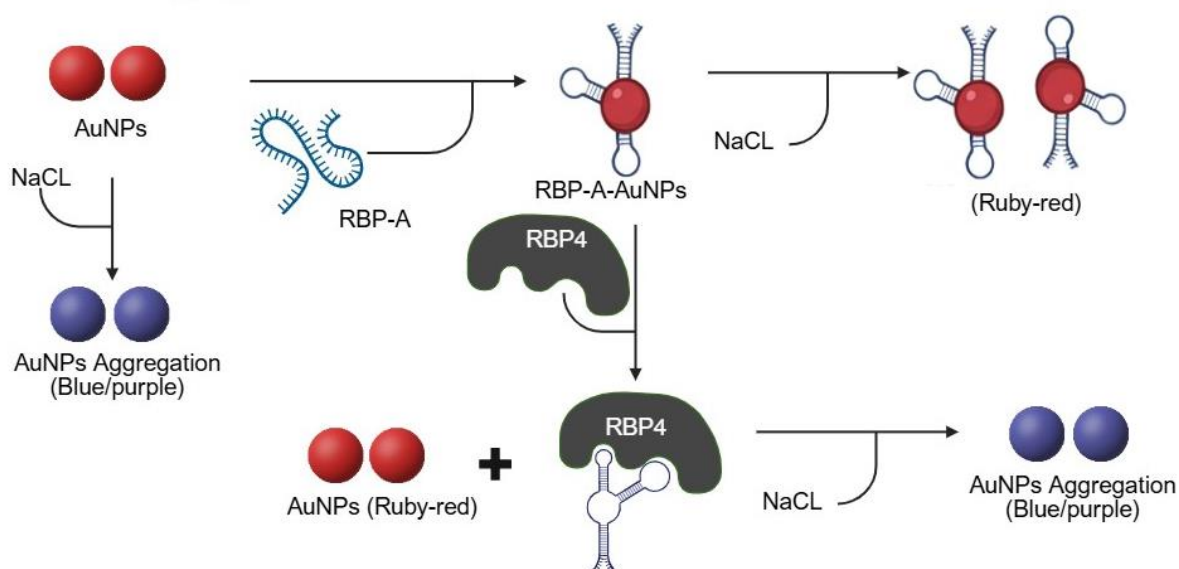
Figure 4 Aptamer-based LFAs. (A) competitive and (B) sandwich assay formats. Adapted from [48].

AuNPs-Based in-Solution Colorimetric Assays. AuNPs typically appear red in solution but can change from red to purple, blue or black when changes occur in their refractive index or when they aggregate [86]. The salt-induced aggregation mechanism of AuNPs is fundamental to many colorimetric detection strategies. Citrate-reduced AuNPs are stable in solution because electrostatic repulsion between the negatively charged citrate ions maintains their dispersion. In the absence of stabilizing agents, such as aptamers, adding salt (NaCl) reduces electrostatic repulsion between AuNPs, promoting aggregation. This results in a shift in LSPR, leading to a visible color change from red to blue. In aptamer-functionalized systems, the presence of target molecules can induce conformational changes in the aptamer, reducing its ability to attach and stabilize AuNPs, thereby causing AuNPs instability in the presence of NaCl. This mechanism enables visual detection of target analytes [87, 88]. The color change can be visually detected with the naked eye and indicate alterations in NP size and interparticle distance, which affect their light-absorption properties. This phenomenon has been widely explored for use in colorimetric detection assays for both chronic [89] and infectious diseases [69].

In-solution colorimetric assays have been applied for the rapid detection of food-borne bacteria [90] and for the diagnosis of coronavirus disease 2019 [91]. For example, the binding of graphene-oxide-coated AuNPs immunosensor to *S. typhimurium* or *E. coli* as target analytes resulted in visible color change within 5 minutes. The limit of detection (LoD) for *S. typhimurium* was 10^3 CFU/mL [90]. Similarly, AuNPs-based immunosensor have demonstrated considerable promise for the rapid detection of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [91]. The interaction between SARS-CoV-2 antigens (or virus particles) and antibodies resulted in a color change to blue within 5 minutes. The LoD for SARS-CoV-2 antigens and viral particles were reported to be 1 ng/mL and 1000 particles/mL, respectively [92].

The dispersion and aggregation behavior of AuNPs generally follows two strategies, as illustrated in Figure 5. The first strategy (Figure 5A) relies on the displacement of folded aptamers from the AuNPs surface. For example, an aptamer-AuNPs biosensor was developed to detect retinol-binding protein 4, a biomarker for the early diagnosis of type 2 diabetes mellitus, resulting in the detachment of the aptamer from the AuNP surface [93]. In the second strategy (Figure 5B), two single-stranded aptamers that bind to different regions on the target analyte were conjugated to AuNPs. On binding to the analyte, they formed a sandwich structure that brings the AuNPs into proximity [93]. In both strategies, changes in the interparticle spacing led to AuNP aggregation in the presence of NaCl, resulting in a visible color change from red to purple or blue [94]. Using this principle, several AuNP-based aptasensors have been developed for the detection of food- and water-borne pathogens across various matrices, including water, milk, and meat samples.

A Competitive aggregation



B Non-competitive agglomeration

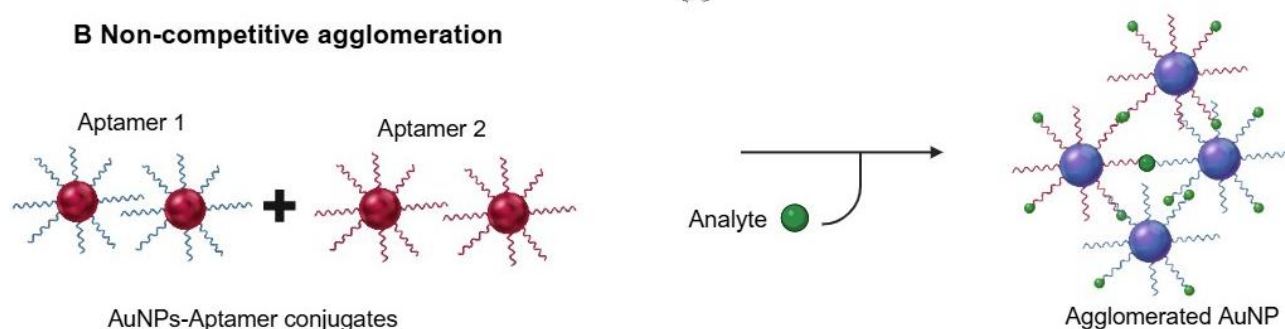


Figure 5 In-solution colorimetric aptasensors for the detection of analytes using folded and unfolded aptamers. (A) Aptamers-AuNP conjugates are protected from salt-induced aggregation. The color change to blue or purple represents a positive test. (B) A sandwich aptasensor in which two aptamers bind to different aptatopes in a sandwich format, bringing the AuNPs into proximity and making them susceptible to salt-induced aggregation. (A) is reprinted with permission from MDPI [93], and (B) is adapted from [48].

A two-step label-free aptasensor by Kim and colleagues further demonstrated the simplicity of aptamer-based detection systems. In their strategy, aptamers were first incubated with the samples, and the resulting supernatant was subsequently used for testing. In a negative sample, the aptamers bind to the AuNPs, thereby protecting them from salt-induced aggregation. However, if the target bacterium is present in the sample, it binds to the aptamers, leaving the AuNPs unprotected and susceptible to aggregation. This strategy does not need complex chemical modifications; however, centrifugation is required during the preparation step, which may limit its use in low-resource areas [95]. Nevertheless, the aptamer-AuNPs-based assays remain promising tools for on-site testing by end users. A comparison of representative studies, including detection limits, assay time, multiplexing capability, and validation in real samples, is presented in Table 3. AuNPs-based aptasensors demonstrated rapid assay time (10-30 minutes) and low LoDs ($57\text{-}10^5$ CFU/mL) across multiple food matrices, highlighting their potential for rapid on-site detection of food- and water-borne pathogens.

Table 3 Comparison of representative aptamer-AuNPs-based LFA and colorimetric assays for the detection of food- and water-borne pathogens.

Target Pathogen	Detection Strategy	Sample Matrix	Sample Type	LoD	Assay Time	Multiplexing	Real Sample Validation	Reference
<i>Salmonella typhimurium</i>	Aptamer-AuNPs LFA	Milk	Spiked	4.1×10^2 CFU/mL	~15 min	No	Yes	[96]
<i>Salmonella typhimurium</i>	Aptamer-AuNPs LFA	Mixed food samples	Spiked	10^5 CFU/mL	~20 min	No	Yes	[97]
<i>Salmonella typhimurium</i>	Multiplex aptamer-AuNPs LFA	Milk, chicken, frozen foods	Spiked (LoD), Real (validation)	10^3 CFU/mL	10 min	Yes	Yes	[85]
<i>Escherichia coli</i> O157:H7	Multiplex aptamer-AuNPs LFA	Milk, chicken, frozen foods	Spiked (LoD), Real (validation)	10^4 CFU/mL	10 min	Yes	Yes	[85]
<i>Staphylococcus aureus</i>	Multiplex aptamer-AuNPs LFA	Milk, chicken, frozen foods	Spiked (LoD), Real (validation)	10^4 CFU/mL	10 min	Yes	Yes	[85]
<i>Pseudomonas aeruginosa</i>	Aptamer-antibody hybrid AuNPs LFA	Poultry meat	Spiked	$\sim 10^2$ CFU/mL	~15 min	No	Yes	[98]
<i>Leptospira interrogans</i>	Aptamer-AuNPs colorimetric assay	Water	Spiked	57 CFU/mL	~30 min	No	Yes	[99]

Note: LoD values are reported as described in the original studies and may represent measurements obtained from spiked or real samples.

The performance of the aptamer-conjugated AuNPs-based detection system is strongly influenced by several experimental parameters, which affect both aptamer functionality and AuNPs behavior. Factors such as pH play a critical role in maintaining the structural conformation of aptamers; changes in pH conditions may disrupt folding and reduce binding affinity towards target molecules. Similarly, ionic strength, particularly salt concentration, directly affects AuNPs stability, which is used in AuNPs-based colorimetric assays. Increased salt concentration can induce aggregation of AuNPs, leading to a visible color change from red to blue, while optimal ionic conditions are required to maintain assay stability and sensitivity [100]. Temperature also influences both the stability of the aptamer-target interaction and the kinetics of the assay, potentially affecting detection efficiency under varying environmental conditions. In addition, the concentrations of aptamers and AuNPs must be carefully optimized, as insufficient probe density may lead to weak signal generation. In contrast, high concentrations can promote non-specific interactions and reduce assay specificity. Furthermore, intrinsic NP properties, including size and surface characteristics, significantly impact optical behavior and detection sensitivity [101]. Collectively, these factors highlight the importance of optimization of experimental conditions to ensure reliable and reproducible performance of aptamer-AuNPs-based colorimetric assays [102].

Despite these advances, several opportunities remain for further improving the practical implementation of aptamer-AuNPs-based detection systems. Future research should focus on the integration of smartphone-based detection platforms and artificial intelligence (AI)-driven data analysis to enable real-time monitoring and improved diagnostic accuracy, particularly in resource-limited settings [103, 104]. In addition, nanozyme-based signal amplification strategies offer a promising approach to enhance assay sensitivity and stability, thereby addressing limitations associated with conventional enzyme-based systems and expanding the capabilities of colorimetric detection [105]. The development of multiplexed lateral flow platforms capable of simultaneously identifying multiple pathogens will further improve diagnostic efficiency and outbreak response capacity [106]. However, despite these advances, challenges related to standardization, scalability, and regulatory approval must be addressed to facilitate the successful translation of these technologies from laboratory research to real-world applications, especially at PoC and field environments [107]. Addressing these gaps will be essential for the effective deployment of aptamer-AuNPs-based diagnostic systems and for strengthening global food and water safety, especially in LICs.

4. Conclusion

Food security remains a major global challenge, particularly in developing countries. This issue emanates from multiple factors, with bacterial contamination being one of the most prominent contributors. Food- and water-borne pathogens have substantial health and economic impacts, leading to numerous outbreaks and worldwide fatalities. To address this challenge, PoCT strategies are essential for the early identification of contamination sources, thereby preventing the spread of pathogens and reducing the likelihood of outbreaks. AuNPs-based colorimetric assays have gained considerable attention as promising diagnostic tools capable of overcoming many limitations associated with conventional detection methods. These systems have strong potential for deployment at PoC, allowing end users to perform rapid and accessible testing. The successful development and implementation of such systems could enable the detection of contamination

before food and water products reach consumers. Furthermore, the integration of AuNPs with aptamer-based recognition elements may facilitate the development of cost-effective diagnostic platforms for LICs. These technologies could therefore play an important role in improving food and water quality monitoring and enhancing consumer safety.

Acknowledgments

Yandiswa Mabhude's MSc Nanoscience degree was sponsored by the DSTI-funded National Nanoscience Postgraduate Teaching and Training Platform (2022-2023), and the UWC Nanotechnology Platform.

Author Contributions

Yandiswa Mabhude: Formal analysis and investigation, Writing - original draft preparation, Funding acquisition; Adewale Oluwaseun Fadaka: Conceptualization, Supervision, Methodology, Resources, Writing - review and editing; Kwazikwakhe Bethuel Gabuza: Methodology, Resources, Writing - review and editing; Samantha Meyer: Conceptualization, Supervision, Methodology, Writing - review and editing; Abram Madimabe Madiehe: Formal analysis and investigation, Methodology, Resources, Writing - review and editing; Mervin Meyer: Formal analysis and investigation, Methodology, Resources, Writing - review and editing; Nicole Remaliah Samantha Sibuyi: Conceptualization, Supervision, Methodology, Resources, Writing - review and editing, Funding acquisition.

Funding

The study was also supported by Mintek Science Vote grant number AM50.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

Data sharing is not applicable to this article as no data was generated or analyzed in this study.

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