

Review

Recent Advances in Nanomaterials for Enhanced Colorimetric Detection of Viruses and Bacteria

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Abstract: The increasing prevalence of pathogen outbreaks underscores the urgent need for rapid, accurate, and cost-effective diagnostic tools. Colorimetric detection has gained significant attention among the available techniques due to its simplicity, portability, and potential for point-of-care applications. The nanomaterial-based colorimetric detection field continues to evolve, with innovations focusing on improving sensitivity, specificity, robustness, cost-effectiveness, and friendly analysis. Additionally, efforts to address limitations, such as stability and environmental impact, pave the way for more sustainable and reliable diagnostic solutions. This review highlights recent advances in nanomaterials for colorimetric pathogen detection in the last five years.

Keywords: gold nanoparticle; aggregation; LSPR; LFA; virus; bacteria

1. Introduction

Infectious diseases caused by pathogenic microorganisms, including food-borne pathogens such as *Clostridium botulinum*, *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*; water-borne pathogens like *Vibrio cholerae*, *Cryptosporidium parvum*, and *Giardia lamblia*; and nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and vancomycin-resistant *Enterococcus* (VRE); or even others caused by viral pathogens, such as influenza, Zika virus, coronaviruses (e.g., SARS-CoV, MERS-CoV, and SARS-CoV-2), dengue virus, and hepatitis viruses, continue to pose a growing threat to public health and the global economy [1,2]. These infections not only lead to significant morbidity and mortality but also place an immense burden on healthcare systems, disrupt economic activities, and compromise societal resilience [3]. Emerging viral outbreaks such as COVID-19 and the Zika pandemic have further demonstrated the devastating global impact of viral diseases, causing widespread social and economic disruptions [4,5]. Then, the necessity of monitoring viruses and bacteria epidemiological profiles assisted by computational predicting and new low-cost detection technologies is requisite to countries' equity access towards mitigating worldwide emergencies caused by pathogenic agents.

Various conventional approaches to detecting and identifying pathogenic bacteria and viruses, such as culture and colony-counting methods, enzyme-linked immunosorbent assays (ELISA), and polymerase chain reaction (PCR)-based techniques, have been widely developed and employed in the field [6–10]. However, such gold-standard traditional methods often require specialized laboratories, skilled personnel, and extended turnaround



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times, underscoring the urgent need for diagnostic tools that are rapid, sensitive, cost-effective, and easily deployable in diverse settings. Apart from effectiveness, these methods are also time-consuming, labor-intensive, and reliant on specialized and expensive equipment, limiting their accessibility and practicality in resource-constrained settings. The World Health Organization has outlined the ASSURED criteria—Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable to end-users—as a benchmark for developing practical diagnostic tools [11]. Numerous innovative diagnostic platforms have emerged to address these requirements, including optical and electrical sensors, microfluidic devices, DNA microarrays, and nuclear magnetic resonance tools [12]. Among these, colorimetric biosensors are favored for their simplicity and ability to align with the “ASSURED” criteria. Rapid detection of pathogens is critical for controlling their spread, enabling timely treatment, and implementing practical governmental public health actions.

Colorimetric biosensors have emerged as powerful tools in advancing detection technologies, mainly to detect and analyze color changes triggered by interactions with the target analyte [13–15]. This visual response enables rapid, preliminary assessments with the naked eye, making the approach highly desirable for rapid screening. For example, gold nanoparticles, widely used in colorimetric biosensors, exhibit a distinctive color shift from red to blue upon aggregation, a property harnessed for detecting DNA hybridization and target protein interactions [16]. Compared to other detection methods, the colorimetric approach offers significant advantages, including cost-effectiveness, simplicity, and portability. Notably, this technique can be applied to diverse sample types, such as solids or liquids, without interference from reference materials that may degrade over time. Furthermore, colorimetric methods are particularly effective in detecting specific analytes in challenging scenarios, such as environmental pollutants and trace contaminants in food safety applications [17]. These attributes underscore the versatility and practicality of nanomaterial-based colorimetric biosensors in various applications.

Nanomaterials are increasingly utilized in colorimetric analysis due to their exceptional ability to enhance detection sensitivity and specificity [18–20]. Their high surface-to-volume ratios allow for more substantial interactions with analytes, while their tunable optical properties enable precise control over colorimetric responses. Additionally, nanomaterials often exhibit catalytic activities that amplify the visual signals of chromogenic reactions, making it easier to detect even trace amounts of pathogen biomarkers. These properties make nanomaterials ideal for developing rapid, accurate, and cost-effective diagnostic tools, particularly in point-of-care settings where quick and reliable results are critical for effective disease management and outbreak control.

This review focuses on recent breakthroughs in nanomaterial-based colorimetric detection of pathogens, emphasizing innovative strategies rather than providing a comprehensive field survey. We highlight key advancements leveraging the unique properties of nanomaterials for colorimetric detection, categorizing them into three primary mechanisms: aggregation-based detection, plasmonic effects, and dual/multiplexed detection platforms. Recently, research has emphasized the development of colorimetric sensors based on the distinctive optical properties of gold nanoparticles (AuNPs), such as those for pathogen detection [21] and bacterial contamination monitoring in food, water, and environmental safety [22,23]. Other studies have investigated colorimetric strategies for identifying pathogenic viruses, integrating carbon allotropes and inorganic/organic nanomaterials for virus sensing [24,25], and detecting airborne pathogens. We showcase the versatility and potential of new virus and bacteria diagnostic methodologies, focusing on the most impactful developments from the last five years. Additionally, we provide insights into future

directions, emphasizing the need for advanced nanomaterials to enhance the sensitivity, specificity, and practical applicability of colorimetric biosensors.

2. Colorimetric Detection Based on Aggregation

Since 1990, nanomaterials have garnered significant interest in the colorimetric field due to their unique properties, distinguishing them from conventional bulk materials [26–28]. Their integration into the development of biosensors has emerged as a highly dynamic area of research, driven by their remarkable sensitivity, exceptional selectivity, high surface-area-to-volume ratio, and nanoscale dimensions. The pioneer methodology describes detection based on the aggregation of nanomaterials in the presence of pathogens, with the process being influenced by both the size of nanomaterials and the degree of their aggregation [29,30]. This phenomenon arises from shifts in the absorbance spectrum within the visible light range, allowing its quantification after easily eye-naked color change detection. This visually detectable transformation offers a straightforward and practical platform for colorimetric detection, enabling the rapid and efficient identification of pathogenic bacteria and viruses. Such mechanisms revolutionize aggregation-based systems in developing accessible and user-friendly diagnostic tools for various applications [31], mainly using gold nanoparticles due to their interesting features.

Gold nanoparticles (AuNPs) are widely regarded as the most versatile and practical tools in developing colorimetric detection systems for pathogen-specific antibodies and proteins [32–35]. Their unique optical properties, particularly the ability to exhibit distinct color changes upon aggregation, make them ideal for visual detection. When antibodies bind to their corresponding antigens on the surface of a pathogen, this binding triggers the aggregation of gold nanoparticles, leading to a measurable color shift. Gold-like aggregation is primarily due to the collective behavior of the nanoparticles, which alter their surface plasmon resonance, a phenomenon that underlies the visible color change [36]. This simple yet highly sensitive mechanism has positioned gold nanoparticles as a cornerstone in biosensor technology, offering a rapid, cost-effective, and highly accurate approach for detecting pathogens in various diagnostic applications. Our group has studied the functionalization of gold nanoparticles with different biomolecules easily and practically [36–41]. These studies highlighted challenges in this area, particularly concerning the stability and activity of conjugated proteins. Aggregation-based colorimetric detection using AuNPs employs a variety of methodologies to induce and control them, leveraging specific molecular interactions and chemical modifications. Classic AuNP colorimetric assays typically rely on monitoring color changes in a solution to determine a target analyte's concentration, providing a straightforward and low-cost approach lacking the need for complex sample pretreatment or intricate instrument operation [36]. The fundamental design strategy often hinges on aggregation or anti-aggregation phenomena, which result in noticeable color shifts that can be easily observed. EDC/NHS (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) coupling remains a widely employed technique in colorimetric methodologies, particularly for functionalizing AuNPs. This method facilitates the covalent attachment of biomolecules, such as antibodies, proteins, and aptamers, to the surface of AuNPs. The process involves EDC activating carboxyl groups on the target biomolecule, forming an active intermediate that reacts with NHS to create a stable NHS ester. This ester can then efficiently bind to amine groups on the surface of AuNPs, forming a robust amide bond. This coupling increases the likelihood of successful biomolecule attachment, thus improving the sensitivity and selectivity of the colorimetric assay. The EDC/NHS method is particularly advantageous in colorimetric detection due to its simplicity, effectiveness under mild conditions, and ability to preserve the bioactivity of the attached molecules. This technique functionalizes AuNPs by attaching biomolecules, such

as antibodies, aptamers, or peptides, to their surfaces, enhancing their utility in various biomedical and biosensing methodologies [42–45]. This approach creates a stable and specific interface for target recognition.

Another standard methodology utilizes enzymatic reactions to induce aggregation. Enzymes such as horseradish peroxidase (HRP) or urease catalyze reactions that produce charged species, altering nanoparticle electrostatic interactions and triggering aggregation. These enzyme-mediated methods are highly efficient and sensitive, and specific enzyme-based assays resembling ELISA have been developed to detect bacteria and viruses, encompassing gram-negative and gram-positive bacteria and RNA and DNA viruses. These assays predominantly utilize nanomaterials exhibiting peroxidase-like activity, including pristine, composite, or derivative forms of silver nanoclusters [46], gold nanoparticles [47], iron oxide nanoparticles [48], graphene quantum dots [49], cobalt oxide nanoparticles [50], manganese dioxide nanoflowers [51], and metal-organic frameworks [52]. The bacterial targets cover *Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Enterobacter sakazakii*, *Yersinia enterocolitica*, and *Burkholderia pseudomallei* (gram-negative), along with *Staphylococcus aureus* and *Streptococcus mutans* (gram-positive). Virus detection encompasses RNA viruses such as SARS-CoV-2, HIV, avian influenza A, norovirus (NoV), Zika virus, Rubella virus, measles virus, mumps virus, and respiratory syncytial virus, as well as DNA viruses including hepatitis E virus, porcine circovirus type 2, and human papillomavirus [42–52]. Each methodology exhibits unique detection limits regarding quantification apart from the eye-naked feature and linear ranges, enabling tailored detection for diverse similar pathogens.

DNA hybridization is another highly effective approach for nucleic acid detection, leveraging the unique properties of nanoparticles. In this method, complementary DNA strands functionalized on AuNPs bind selectively to a target DNA sequence, resulting in a well-controlled aggregation of nanoparticles [53]. This aggregation, induced by the hybridization event, can be easily monitored due to the notable color change associated with the shift in the surface plasmon resonance of the gold nanoparticles. The ability of AuNPs to enhance the sensitivity and specificity of DNA detection makes them a powerful tool for applications such as pathogen detection and genetic analysis [54,55]. In another approach, DNA hybridization has been studied as ionic strength or pH change, which can also be utilized to induce the aggregation of gold nanoparticles. For example, introducing high salt concentrations, such as sodium chloride (NaCl), can screen the electrostatic repulsion between AuNPs, allowing them to aggregate in the presence of a specific target molecule [56]. This aggregation is often accompanied by a noticeable color change, which can be easily detected visually. Such strategies are particularly effective in biosensor applications where sensitivity and simplicity are paramount. For instance, in detecting specific proteins or pathogens, adding salts can promote the formation of aggregates when AuNPs are functionalized with antibodies or aptamers that bind to the target. A similar approach is used to detect environmental pollutants, where changes in pH trigger aggregation of functionalized AuNPs, providing a straightforward and rapid detection method. This ionic strength or pH-based aggregation approach is highly suitable for resource-limited settings, as it does not require expensive equipment or complex reagents, making it accessible for field diagnostics and low-cost healthcare applications, demonstrating its broad applicability and efficiency [57–59].

Moreover, nonspecific adsorption has been a significant challenge in colorimetric biosensing, as it can interfere with accurate detection by leading to false-negative results, mainly when pathogens are present at low concentrations. Unwanted interactions between nanomaterials and non-target biomolecules can reduce sensor sensitivity, hinder signal generation, and compromise assay reliability. This issue becomes even more pronounced in

complex biological and environmental samples, where proteins, lipids, and other interfering substances may adsorb onto the sensor surface, masking or weakening the specific detection signal. To address this problem, various strategies have been explored. Researchers have achieved highly selective pathogen recognition by modifying the nanomaterial surface with specific ligands, such as antibodies [42], aptamers [56], or DNA/RNA [52], while reducing background noise from unwanted interactions. Antibodies are widely used due to their strong affinity for viral and bacterial antigens. They are typically immobilized on nanoparticle surfaces through covalent bonding or physical adsorption. However, proper orientation control is crucial to ensure the binding sites remain accessible for target recognition [60,61]. Recently, a low-cost and easy-to-use colorimetric flu virus biosensor was developed using a sandwich assay format. This approach immobilized antibodies onto cotton swabs to rapidly detect influenza A and B viruses. Notably, the biosensor showed no cross-reactivity with non-specific antigens, indicating minimal nonspecific adsorption. Moreover, the method demonstrated stability for over six months at room temperature, making it highly practical for real-world applications [62]. Aptamers have also been explored to minimize nonspecific adsorption. Due to their stability and ability to undergo conformational changes upon binding, aptamer-functionalized nanoparticles enhance pathogen detection while reducing interference from non-target molecules [63]. For instance, a colorimetric biosensor using aptamer-functionalized gold nanoparticles has been successfully employed to detect *Pseudomonas aeruginosa*, demonstrating high sensitivity and specificity [64]. Aptamer-functionalized nanomaterials enhance selectivity while minimizing nonspecific interactions in complex biological samples. Newly, a novel biosensor was developed using aptamer-functionalized polydiacetylene (Apta-PDA) to detect *E. coli* O157:H7, *Salmonella typhimurium*, and *Vibrio parahaemolyticus*. In this approach, the target bacteria were covalently modified onto the surface of magnetic beads (MBs) to form MB–oligonucleotide conjugates, allowing bacterial enrichment while preventing nonspecific adsorption. This method significantly improved detection accuracy and reduced background noise [65]. Another promising approach involves molecularly imprinted polymers (MIPs) that mimic natural receptors [66,67]. MIPs are fabricated by polymerizing functional monomers around a specific virus or bacterial epitope, which is later removed to create highly selective binding sites. When incorporated into colorimetric sensors, MIPs significantly reduce nonspecific adsorption by ensuring that only the target pathogen binds to the sensor surface, improving detection reliability in real-world applications. Zwitterionic materials have gained attention recently due to their exceptional anti-nonspecific adsorption properties [68,69]. These materials carry positive and negative charges, preventing unwanted interactions with non-target molecules while maintaining strong binding specificity for pathogens. A recent study introduced a *Listeria monocytogenes*-specific biosensor utilizing sodium sulfonyl methacrylate (SBMA) polymers. These polymers were photothermally polymerized onto cotton swabs and combined with a *Listeria monocytogenes*-specific aptamer (Apt1) to create SBMA/Apt1 cotton swabs. This innovative design effectively captured and isolated *Listeria monocytogenes* from complex sample matrices, expanding the detection range and offering a promising new strategy for food safety monitoring [70]. Overall, the combination of antibodies, aptamer-functionalized nanomaterials, molecularly imprinted polymers, and zwitterionic materials represents a powerful approach to enhancing the specificity and accuracy of colorimetric biosensors. By integrating these strategies, researchers have minimized nonspecific adsorption and improved pathogen detection in diverse real-world applications. All these methodologies have discussed the complexities of maintaining protein functionality upon conjugation to nanomaterial, emphasizing the need for optimized surface modification techniques to preserve biological activity. These findings underscore the importance of ad-

addressing challenges related to protein conjugation and detection capabilities in developing nanomaterial-based colorimetric applications.

Next, we will focus on recent efforts to enhance colorimetric sensors for visually detecting ultralow analyte concentrations. Researchers have explored various approaches to overcome these challenges, including signal amplification techniques and integrating cutting-edge nanomaterials with remarkable physicochemical properties, particularly plasmonic nanomaterials. Moreover, advancing lateral flow assays (LFAs) have revolutionized rapid and on-site diagnostic applications, providing a cost-effective and user-friendly platform for detecting pathogens and biomarkers. Additionally, dual and multiplexed detection platforms have further enhanced the analytical performance of colorimetric sensors by allowing simultaneous detection of multiple analytes, reducing false positives, and improving overall diagnostic accuracy. As a result, these innovative strategies are paving the way for next-generation colorimetric sensors with superior sensitivity, specificity, and practical applicability in various fields, including medical diagnostics, environmental monitoring, and food safety.

3. Plasmonic Properties for Enhanced Sensitivity

Integrating plasmonic nanoparticles with biomolecules—such as enzymes, antibodies, DNA, or aptamers—has become a cornerstone in developing advanced colorimetric technologies [71]. This integration leverages the unique optical properties of plasmonic nanoparticles, which exhibit strong localized surface plasmon resonance (LSPR) effects. When these nanoparticles bind to specific biomolecules, the local refractive index near the nanoparticle surface changes, leading to detectable shifts in the LSPR signal. This phenomenon enables highly sensitive and label-free detection of various analytes, including pathogens, toxins, and biomarkers [71]. Moreover, the functionalization of plasmonic nanoparticles with selective biomolecules enhances the specificity of the biosensors, allowing for precise targeting of desired analytes. The high specificity of biomolecules for target recognition is enhanced by coupling nanoparticles with biomolecules, enabling the creation of highly sensitive and selective sensing platforms capable of detecting a wide range of biological targets, including pathogens, biomarkers, and environmental contaminants. These hybrid systems have paved the way for medical diagnostics, environmental monitoring, and food safety innovations, offering rapid, accurate, and scalable solutions to pressing challenges in these fields [72,73].

Gold is among the most favored materials for LSPR applications due to its exceptional optical properties, ease of synthesis, chemical and photostability, biocompatibility, and straightforward surface functionalization. AuNPs typically range in size from 1 to 100 nm and are commonly synthesized as colloidal particles dispersed in aqueous solutions [74]. Their functionalized forms exhibit excellent stability and biocompatibility, making them highly effective for detecting analytes in complex biological environments. Leveraging the intrinsic properties of AuNPs enables the design of highly efficient sensing platforms. Their strong optical responses facilitate the development of colorimetric sensors, which offer simple, rapid, and cost-effective solutions for detecting various targets across biomedical, environmental, and industrial domains [74,75]. Compared to other types, one of the most appealing features of colorimetric sensors is their simplicity. The entire analysis process can be performed using only the naked eye, without complex or sophisticated instrumentation. This makes colorimetric systems highly accessible and cost-effective, offering rapid, on-site detection capabilities ideal for resource-limited settings or field applications. The ease of interpretation, based solely on color changes, enhances the practicality and user-friendliness of these sensors for a wide range of detection tasks [76,77].

3.1. Antibody Functionalization

In recent years, combining antibodies with colorimetric localized surface plasmon resonance has significantly advanced pathogen detection, offering rapid and sensitive diagnostic tools. For instance, antibody-conjugated AuNPs have been employed in food safety applications to detect foodborne pathogens. These biosensors recognize specific pathogens, leading to nanomaterial aggregation and a corresponding color change, facilitating the visual identification of contaminants. This method has been applied to detect various bacteria, including *Salmonella enterica* [77], *Escherichia coli* [78], ochratoxin A [79], and *Staphylococcus aureus* [80], demonstrating the potential for rapid on-site testing. Similarly, studies based on antibody-functionalized AuNPs have been utilized to detect oral bacteria. Bacteria binding to these antibodies induces changes in the LSPR signal, has been developed, and authors observed a color change in the solution [81]. This approach allows for the rapid and specific identification of four oral bacterial species (*Aggregatibacter actinomycetemcomitans*, *Actinomyces naeslundii*, *Porphyromonas gingivalis*, and *Streptococcus oralis*) in oral samples, aiding in the diagnosis and management of oral infections. In this context, Marin et al. developed a direct colorimetric detection of *S. aureus* using an aptamer sensor based on LSPR and AuNPs in milk and infant formula [82]. In the assay, *S. aureus* selectively bound to aptamers, depleting them from the test solution. This depletion triggered the aggregation of AuNPs upon adding salt, resulting in a visible color change from red to purple. Under optimized conditions, the assay enabled visual detection of *S. aureus* within 30 min, with detection limits of 7.5×10^4 CFU/mL in milk and 8.4×10^4 CFU/mL in infant formula. Recently, Seele et al. presented the development of a rapid, cost-effective diagnostic test for tuberculosis (TB) by detecting *Mycobacterium* biomarkers from non-sputum-based samples [83]. Two AuNP-based rapid diagnostic tests, designed as lateral flow immunoassays, were developed to target immunodominant TB antigens: the 6 kDa early secreted antigen target EsxA (ESAT-6) and the 10 kDa culture filtrate protein EsxB (CFP-10). AuNPs were synthesized using the Turkevich method and characterized using a UV-Vis spectrophotometer and transmission electron microscopy (TEM). Kaushal et al. have harnessed metallic nanoparticles' unique optical and plasmonic properties, positioning them as innovative and powerful components for integration into biosensors aimed at bacterial detection [84]. This study developed a hybrid antibody biosensor featuring graphene oxide (GO)-coated gold nanoparticles (AuNPs) for the rapid, specific, and highly sensitive detection of *Escherichia coli* and *Salmonella typhimurium*. The colorimetric assays exhibit an apparent, visible color change within just 5 min upon the binding of the nanosensor to the target bacteria (Figure 1A). This assay is highly convenient and requires no sample pretreatment or specialized training. Zhao et al. developed a versatile, antibody-free bacterial detection platform allowing naked-eye observation [85]. This platform is composed of concanavalin A-modified gold nanoparticles (ConA-AuNPs), vancomycin-modified gold nanoparticles (Van-AuNPs), and polymyxin B-modified Prussian blue nanoparticles (PMB-PBNPs). The platform operates based on the rapid agglutination of bacterial cells induced by concanavalin A. ConA-AuNPs bind to *Escherichia coli* and *Staphylococcus aureus* cells (Figure 1B). This leads to aggregation and a visible color change within 30 min. This color shift occurs due to the alteration of surface plasmon resonance properties of the nanoparticles.

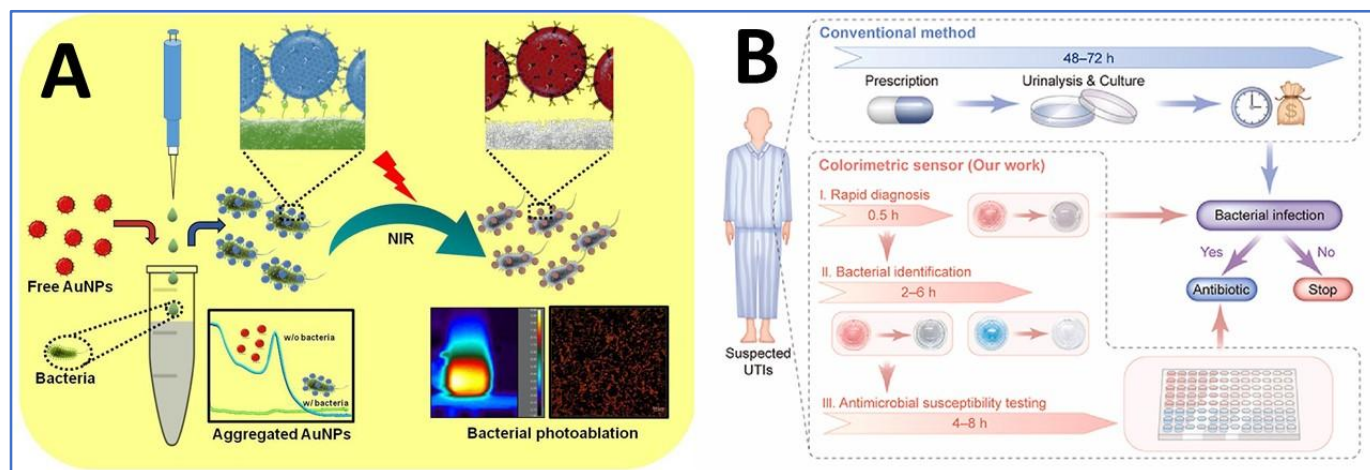


Figure 1. (A) Schematic representation of specific bacterial recognition through antibody conjugated PEG-GO-AuNPs (pegylated graphene oxide coated gold nanoparticles) via colorimetric detection and its photothermal ablation upon NIR irradiation, reproduced with permission [84]. (B) In the clinical diagnosis process for UTIs, physicians prescribe antibiotics based on experience, reproduced with permission [85].

Similar approaches have been employed for virus detection, such as the Canine Distemper virus [36], Dengue [38], PCV-2 [39], Influenza A [46], and others. Two novel strategies have recently paved the way for advanced colorimetric analysis, offering new avenues for sensitive and rapid detection [86,87]. A colorimetric serological assay was developed to detect SARS-CoV-2 IgGs in patient plasma, utilizing short antigenic epitopes conjugated to gold nanoparticles (AuNPs) [86]. The specific bivalent interaction between SARS-CoV-2 antibodies and the epitope-functionalized AuNPs triggers nanoparticle aggregation, leading to a noticeable optical shift in the AuNPs' plasmonic characteristics within 30 min of antibody addition. By co-immobilizing two epitopes, the assay's sensitivity was significantly improved over single-epitope AuNPs, achieving a limit of detection of 3.2 nM, corresponding to IgG levels in convalescent COVID-19 patients. This strategy was applied to preserve its sensing capability in human plasma to enhance the assay's stability. Zhang et al. introduced a methodology that integrates CRISPR/dCas9 with the localized surface plasmon resonance (LSPR) of gold nanoparticles (AuNPs) [87]. Their approach involves designing a dual protein corona-mediated detection platform capable of simultaneously enabling rapid point-of-care (POC) testing and single-molecule counting of nucleic acids in a one-pot, one-step process. As a result, targets as low as 100 aM can be visually detected within just 30 min, making this platform highly suitable for rapid point-of-care (POC) applications and the early screening of emerging epidemics. Furthermore, the exceptional LSPR properties of AuNPs enhance the light-scattering signal during target-induced aggregation, allowing the aggregated AuNPs to be visualized as diffraction-limited spots under confocal microscopy.

In a recent study, D'Amato et al. designed a biosensor to detect the Zika virus (ZIKV) non-structural protein 1 (NS1), which utilizes gold nanoparticles (AuNPs) functionalized with monoclonal antibodies, coupled with dynamic light scattering (DLS) for detection [88]. This approach allows for sensitive and specific identification of the NS1 protein, providing an effective tool for ZIKV detection. During the experiments, the high ionic strength medium caused particle aggregation in the absence of the protein, demonstrating a detection limit of $0.96 \mu\text{g mL}^{-1}$. The technique was also specific for detecting the Zika virus and did not show cross-reactivity with the DENV2 and SARS-CoV-2 spike proteins that were also tested. Another methodology constructed a colorimetric immunosensor for detecting SARS-CoV-2 infection [89]. This method utilizes a localized surface plasmon resonance

(LSPR) sensor based on a silver nanotriangle (AgNT) array functionalized with human angiotensin-converting enzyme 2 (ACE2) protein, designed for swift coronavirus detection and validated with SARS-CoV-2. The limits of detection for the spike receptor-binding domain (RBD) protein, CoV NL63 in buffer, and untreated saliva are 0.83 pM, 391 PFU/mL, and 625 PFU/mL, respectively, with a detection time of less than 20 min, providing a straightforward and effective detection method.

3.2. Aptamer-Functionalized

Aptamer-based LSPR colorimetric detection has emerged as a powerful and highly sensitive approach for identifying pathogens [90,91]. Aptamers—short, single-stranded DNA or RNA sequences with high specificity for target pathogens—serve as molecular recognition elements, enabling precise and selective detection. Upon interaction with a pathogen, aptamer-functionalized AuNPs undergo conformational changes or aggregation, leading to a measurable shift in LSPR signals or a visible color change. This colorimetric response allows for rapid, label-free, and equipment-free detection, making it ideal for point-of-care (POC) applications and field diagnostics. Recent advancements in this methodology have significantly improved detection sensitivity, enabling the identification of pathogens at ultralow concentrations. For instance, Zhan et al. present a simple and efficient colorimetric detection platform for bacterial identification using silver (Ag) nanoplates as a chromogenic substrate [92]. The method leverages aptamers' high specificity and affinity, along with the catalytic activity of catalase, which hydrolyzes H_2O_2 to etch Ag nanoplates. By incorporating catalase into a sandwich structure based on a dual-aptamer recognition strategy, the presence of bacteria is translated into a detectable LSPR peak shift and a visible colorimetric change. This approach enables rapid, naked-eye detection of *S. aureus* at concentrations as low as 60 CFU/mL, benefiting from the combined sensitivity of the streptavidin-biotin system and the inherent plasmonic properties of Ag nanoplates. Deb et al. harness the localized surface plasmon resonance (LSPR) properties of gold nanoparticles (AuNPs) to develop a point-of-care aptasensor, facilitating the rapid and reliable screening of urinary tract infection (UTI) samples with high sensitivity and specificity [93]. This sensor exhibits distinct absorbance changes in the visible spectrum upon interaction with the target pathogen, ensuring high specificity and sensitivity. In this study, we demonstrate the precise detection of *Klebsiella pneumoniae* with a limit of detection (LoD) as low as 3.4×10^3 CFU/mL. This study demonstrates the quantification of *Klebsiella pneumoniae* in human urine samples. Additionally, the developed prototype has the potential to identify the effectiveness of specific drugs, helping to determine whether a pathogen is susceptible or resistant due to mutations that contribute to antimicrobial resistance (AMR). Arani et al. focus on developing a colorimetric *Vibrio cholerae* aptasensor using gold nanoparticles (GNPs) and the localized surface plasmon resonance (LSPR) technique [94]. A specific DNA aptamer was selected and validated through bioinformatics analysis and molecular docking simulations to ensure strong binding affinity to the outer membrane protein U (OMP U) of *V. cholerae*. The aptamer's effectiveness in bacterial detection was assessed by monitoring the aggregation or dispersion of GNPs in the presence of NaCl. The final evaluation was conducted using both colorimetric analysis and LSPR spectral measurements. Molecular docking results confirmed that the selected aptamer demonstrated high sensitivity and specificity in detecting OMP U on the surface of *V. cholerae* in suspension.

A colorimetric aptasensor was developed to detect oxytetracycline (OTC) based on the aggregation of aptamer-functionalized gold nanorods (AuNRs) [95]. The synthesized AuNRs were thoroughly characterized and functionalized with a specific DNA oligonucleotide aptamer, which generated an optical signal upon binding to the OTC

antibiotic (Figure 2A). The longitudinal surface plasmon resonance of AuNRs exhibited a concentration-dependent decrease within the linear range of 0.1 nM to 100 nM, with a detection limit as low as 0.04 nM. This proposed method is simple, highly sensitive, and selective, making it a promising approach for OTC detection. This approach enables label-free detection, eliminates the need for complex instrumentation, and offers tunable sensitivity by optimizing aptamer sequences and nanoparticle properties. Aithal et al. employed aptamers to detect SARS-CoV-2 [96]. In this assay, AuNPs were functionalized with specific aptamers targeting the spike membrane protein of SARS-CoV-2 (Figure 2B). An agglomeration assay employing nanoprobe detects elevated concentrations of spike protein in the buffer by measuring the absorbance spectra of the samples. The critical coagulant salt concentration, which triggers agglomeration, is a key metric for assessing spike protein binding with the aptamer, increasing proportionally with spike protein concentration. The technique was validated using plasmon absorbance spectra, and the detection limit was 3540 genome copies/ μ L of inactivated SARS-CoV-2. The nanoprobe can detect as few as 3540 genome copies/ μ L and higher concentrations of inactivated SARS-CoV-2 virus. With further validation using real-world samples, both methodologies could be adapted into a user-friendly, inexpensive, and straightforward approach. Aptamer-based colorimetric methods enable real-time monitoring and offer adaptability across diverse environmental and clinical applications, enhancing their versatility and effectiveness in colorimetric assays.

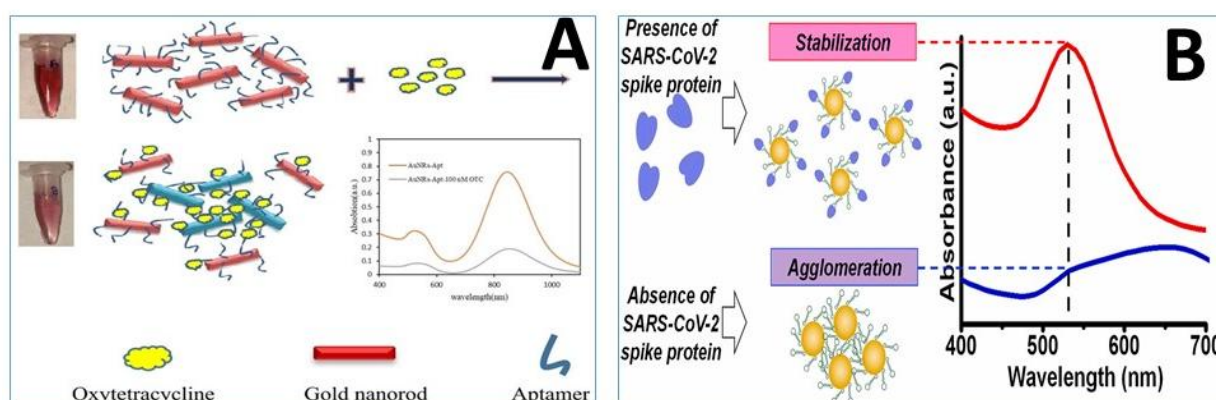


Figure 2. (A) The presence of OTC resulted in AuNRs/aptamer assembly and led to different conjugation and spectroscopic changes, produced with permission [95]. (B) Schematic illustrating the principle of the SARS-CoV-2 test. Nanoprobes are AuNPs functionalized with aptamers in an aqueous suspension. When the SARS-CoV-2 spike protein is absent from the colloid, adding the coagulant Salt M neutralizes surface charges on the nanoprobe, inducing their agglomeration. Nanoprobes with spike protein bind with aptamers and resist agglomeration, which depends on the extent of this binding. Protein binding provides additional charge to the nanoparticle, enhancing steric stabilization. Reproduced with permission [96].

3.3. Other News Approaches

Several LSPR strategies have been developed for the colorimetric detection of pathogens in biological samples, each offering unique advantages for specific applications. One new methodology utilizes chromogenic substrate-mediated catalytic activity, where enzymes produced by the pathogens catalyze a reaction that results in a colorimetric shift, indicating the presence of the target [97]. Another study presents a simple, cost-effective digital microfluidic (DMF) platform combined with colorimetric loop-mediated isothermal amplification (LAMP) for on-site disease diagnosis, visible to the naked eye [98]. The DMF chip features four parallel units, enabling the simultaneous detection of multiple genes and samples. The platform's analytical performance was demonstrated by detecting *Enterocytozoon hepatopenaei*, infectious hypodermal and hematopoietic necrosis

virus, and white spot syndrome virus genes in shrimp. Its sensitivity was comparable to microfluidic-based LAMP assays using other point-of-care testing (POCT) devices, such as centrifugal discs, for the same targets. Additionally, the device's straightforward chip structure and high flexibility for multiplex analysis make it a promising tool for broader POCT applications. Zhao et. Al. presents a particular visual bacterial sensing assay based on a surface plasmon resonance (SPR)-enhanced peroxidase (POD) mimetic [99]. The POD mimetic, designed using platinum (Pt) nanoparticles asymmetrically decorated on Au/TiO₂ magnetic nanotubes (Au/Pt/MTNTs), leverages the intrinsic photocatalytic activity of TiO₂ and the limited transport depth of light. These asymmetric nanotube-localized surface plasmon resonance (LSPR) effect significantly enhances the generation of hot electrons, which are efficiently transferred to Pt and MTNTs, thereby considerably improving catalytic performance. Using *Staphylococcus aureus* (*S. aureus*) as a model for Gram-positive bacteria, the assay exploits the colorimetric reaction's dependence on the POD mimetic's active sites, enabling sensitive and selective bacterial detection.

More recently, machine learning-assisted colorimetric sensor arrays have emerged as a powerful tool for enhancing detection accuracy and sensitivity. Traditionally, colorimetric sensors for food applications have utilized linear regression to correlate colorimetric data with bacterial concentration for detecting foodborne pathogens. Researchers have identified linear relationships between bacterial concentration and various colorimetric parameters depending on the data type, enabling quantitative microbial analysis [100]. Yang et al. present a machine learning-assisted colorimetric sensor array that leverages ligand-functionalized Fe single-atom nanozymes (SANs) for microorganism identification at the order, genus, and species levels [101]. The array was constructed using SAN Fe₁-NC functionalized with four distinct recognition ligands, generating unique microbial identification fingerprints. This platform can identify more than 10 microorganisms in UTI urine samples in less than one hour. Diagnostic accuracy of up to 97% was achieved in 60 UTI clinical samples, holding great potential for translation into clinical practice applications. Another methodology involves the development of a microfluidic biosensor for the rapid and sensitive detection of *Salmonella*, utilizing nanoflowers to amplify the biological signal while enabling automated operations [102]. A smartphone app equipped with a saturation calculation algorithm processes the captured images for analysis. The detection process begins with immune magnetic nanoparticles capturing *Salmonella* from the sample. These nanoparticles are then mixed with immune MnO₂ nanoflowers (NFs) and incubated in a spiral micromixer, facilitating the formation of MNP-bacteria-MnO₂ sandwich complexes. These complexes are subsequently magnetically captured in a dedicated separation chamber within the microfluidic chip, allowing efficient and precise bacterial detection.

4. Point-of-Care Testing Based on Colorimetric Sensors

Colorimetric sensing is a leading point-of-care testing (POCT) method across diverse applications [103]. Over the past five years, more than 500 research papers have been published on this topic, reflecting its growing significance. To streamline traditionally complex detection processes, various POCT devices have been developed, integrating both sensing and readout mechanisms through visible color changes, making them highly practical. These devices are designed for ease of use, allowing non-specialists to operate them with minimal training. By leveraging nanostructures, colorimetric POCT platforms enhance detection accuracy through direct visual assessment or smartphone-based analysis. The following sections explore two key types of colorimetric POCT devices: lateral flow assays and microfluidic chips.

Lateral flow assay (LFA)-based colorimetric sensors offer a rapid, cost-effective, and user-friendly approach for detecting various pathogens [104–109]. These sensors leverage

the principles of capillary action and antibody-antigen or aptamer-target interactions to produce a visible color change, enabling easy result interpretation without the need for specialized equipment. Recently, Tian et al. developed polydopamine (PDA)-coated dyed cellulose nanoparticles (dCNPs@P) with tunable colors as probes for multiplex lateral flow assays (mLFAs) [108]. The cellulose nanoparticles (CNPs) were synthesized with uniform spherical shapes and adjustable sizes, ensuring consistency and adaptability. The dCNP@P-based mLFAs demonstrated successful application in detecting multiple mycotoxins in cereals and measuring inflammatory biomarkers to distinguish between viral and bacterial infections. These assays exhibited high specificity and accuracy, outperforming gold nanoparticle-based tests in sensitivity and reliability. Another LFA was developed to detect detection of *Salmonella typhimurium* using an aptamer-based assay [109]. This platform effectively distinguishes relevant color changes with high accuracy. The devices incorporate gold-decorated polystyrene microparticles functionalized with *S. typhimurium*-specific aptamers (Ps-AuNPs-ssDNA), achieving a detection limit of 10^2 CFU mL⁻¹ in buffer solutions and 10^3 CFU mL⁻¹ in romaine lettuce samples.

Lateral flow assay (LFA) devices have also been extensively applied for the colorimetric detection of viruses due to their simplicity, rapid response, and cost-effectiveness [110]. These devices are particularly advantageous for point-of-care diagnostics, allowing quick and reliable screening without sophisticated laboratory equipment. In recent years, numerous LFA-based biosensors have been designed to detect various viral pathogens, including influenza [111], Porcine epidemic diarrhea virus (PEDV) and porcine rotavirus (PoRV) [112], dengue [113], Zika [114], and coronaviruses [115]. Recently, Yang et al. developed an advanced colorimetric LFA tool for detecting and monitoring a virus that caused a global outbreak in 2022 [116]. Their approach leveraged antibody-functionalized nanoparticles to enhance detection sensitivity, allowing for rapidly identifying viral antigens within minutes. Such advancements in LFA technology have improved detection limits, enabling the identification of low viral loads with high specificity. The biosensor consisted of a flexible lateral flow immunoassay (LFIA) with strong colorimetric and enhanced fluorescence dual-signal output for the rapid, on-site, and highly sensitive. A nanocomposite of silicon dioxide (SiO₂) and AuNPs was first synthesized and conjugated with an A29L detection antibody (Catalog#40,891-V08E, Sino Biological Inc. (Beijing, China) to provide good stability, strong colorimetric capability, and superior fluorescence intensity. Next, the structure of the LFIA strip was produced by immobilizing goat anti-mouse IgG and the A29L capture antibody on the surface for control and testing. A running buffer with different concentrations of the MPXV A29L protein was employed for the experiments. After a 15-min incubation, the colorimetric signal on the C/T line of the strip was observed visually. Alternatively, the fluorescence signal was captured using a portable fluorometer for more precise quantitative analysis—Figure 3.

The continuous evolution of LFA-based colorimetric detection strategies, including incorporating novel nanomaterials and signal amplification techniques, has significantly broadened their applicability for emerging infectious diseases. Future developments aim to improve multiplexing capabilities, enabling simultaneous detection of multiple pathogens in a single test, thereby strengthening global surveillance and outbreak preparedness. Ventura et al. demonstrate that a colorimetric biosensor based on gold nanoparticle (AuNP) interactions induced by SARS-CoV-2 is an outstanding tool for detecting viral particles in nasal and throat swabs [117]. AuNPs functionalized with antibodies targeting three SARS-CoV-2 surface proteins (spike, envelope, and membrane) undergo a rapid red shift within minutes when exposed to a virus-containing solution. This colorimetric method enables the detection of extremely low viral loads, achieving a sensitivity comparable to real-time PCR. Another article presents an assay of foldable paper strips, utilizing

nucleic acid strand-displacement reactions to detect SARS-CoV. This amplification enables a colorimetric pH-based readout via a smartphone. In a study of 50 throat swab samples, the assay successfully detected both the presence of SARS-CoV and virus-specific mutations with 100% concordance to real-time PCR. They demonstrated that in the future, affordable, portable, and user-friendly viral screening strategies could be rapidly adapted to detect other emerging pathogens, such as the dengue and Zika viruses.

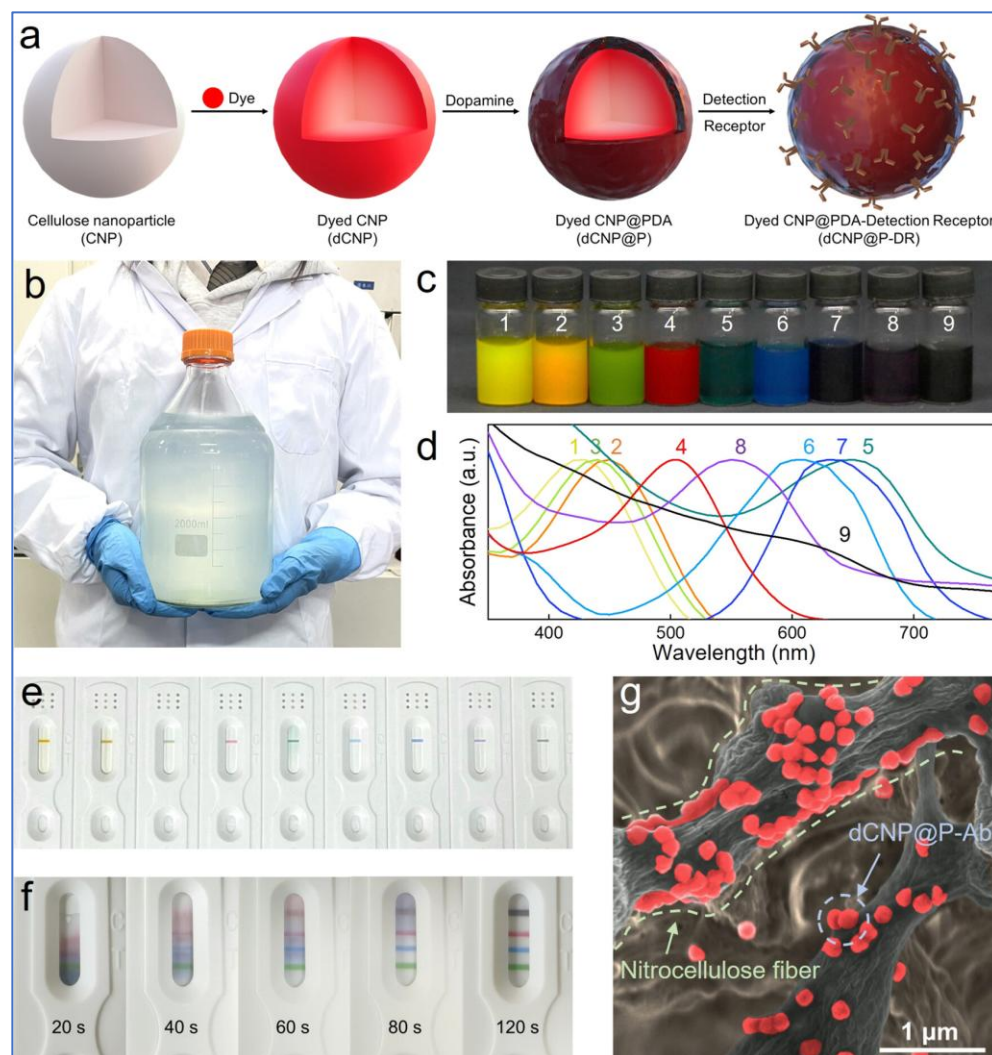


Figure 3. Colored dCNPs@P were used for LFA. (a) Schematic illustration of the synthesis process of dCNPs@P and the conjugation of detection receptor. (b) Photograph of 2 L CNP suspension (1 wt %). (c,d) Colored dCNPs (2 wt %) and the corresponding ultraviolet–visible (UV–vis) absorption spectra. (e) LFA using colored dCNPs@P as probes. (f) Time-elapsed evolution of the colorimetric signals at T lines in an mLFA using colored dCNPs@P as probes. (g) Scanning electron microscope (SEM) image of the T line of a LFA strip capturing dCNPs@P, reproduced with permission [108].

Although LFA is performed on a flat substrate format, it offers several advantages over those in solution, and certain technical challenges remain. One major limitation is the potential for uneven reagent distribution, leading to inconsistent signal intensity and reduced sensitivity. Additionally, surface fouling or nonspecific binding of biomolecules may interfere with accurate detection, necessitating improved surface modification strategies. Another concern is the need for precise fabrication techniques to ensure reproducibility across different sensor batches. Furthermore, optimizing immobilized reagents' stability and shelf life remains a crucial factor for widespread adoption. Addressing these chal-

lenges through advancements in nanomaterials, microfabrication techniques, and surface chemistry will be essential for further improving the reliability and performance.

5. Dual and Multiplexed Detection Platforms

In addition to single nanoparticle-based methods, colorimetric approaches that employ a combination of multiple nanoparticles or combine with other methodologies offer enhanced sensitivity and specificity for pathogen detection [118–122]. Integrating nanoparticles of various materials and sizes makes fine-tuning their optical properties possible, allowing for the simultaneous detection of multiple pathogens in complex samples. Multi-nanoparticle-based systems can generate distinct color changes by leveraging different plasmonic behaviors and nanoparticle interactions. This multi-nanoparticle strategy further improves detection accuracy, enabling a broader range of detectable pathogens and minimizing the chances of false positives or negatives. Furthermore, these systems can be engineered to exhibit synergistic effects, where the combined nanoparticle ensemble enhances the overall performance of colorimetric assays, making them even more robust for point-of-care diagnostic applications.

Magnetic nanomaterials possess great potential for applications in biomedicine. Combining them with biological markers or other nanomaterials while maintaining their magnetic characteristics enhances their sensitivity [123,124]. Recently, plasmonic nanoparticles with magnetic separation, we developed an achromatic colorimetric nanosensor with highly enhanced visual resolution for simultaneous detection of hepatitis E virus (HEV), HEV-like particles (HEV-LPs), norovirus-like particles (NoV-LPs), and norovirus (NoV) [125]. In the presence of one or more pathogens in the sample, magnetic probes separate the respective colors for the detected pathogens magnetically, isolating them from the black color. Therefore, the methodology addresses an easy, rapid, and low-cost sample detection method, depending on the presented color. This well-defined nano platform intelligently integrates dual-modality sensing and magnetic bio-separation, opening a gateway to efficient point-of-care testing for virus diagnostics.

Lately, optically active quantum dots (QDs) encapsulated within an iron oxide (hol-low shell) have been used for virus detection [126]. It presents a dual-modality sensing platform for ultrasensitive virus detection based on V_2O_5 nanoparticle-encapsulated liposomes (VONP-LPs). The sensing mechanism leverages the intrinsic peroxidase-like activity and electrochemical redox properties of V_2O_5 nanoparticles (V_2O_5 NPs). Target-specific antibody-conjugated VONP-LPs and magnetic nanoparticles (MNPs) facilitate virus enrichment via magnetic separation. The bound VONP-LPs are hydrolyzed upon isolation, releasing V_2O_5 nanoparticle NPs, which function as peroxidase mimics and electrochemical redox indicators. This process generates a distinct colorimetric response and a robust electrochemical signal, enabling highly sensitive and specific virus detection. Furthermore, electrochemical analyses demonstrated, through electrochemical impedance spectroscopy, a substantial increase in impedance only in the sample containing Hev-like particles, with a detection limit as low as 1.2 fg/mL. Another methodology provides an innovative dual-signal readout immunochromatography assay (ICA) with colorimetric and fluorescence, which co-enhanced capabilities for the ultrasensitive and flexible detection of the monkeypox virus (MPXV) antigen [127]. This assay utilizes a customized two-dimensional film-like nanotag composed of a molybdenum disulfide core surrounded by multilayered quantum dot shells. Compared to traditional spherical nanolabels, this advanced nanotag offers superior signal responses, a larger reaction interface, and enhanced stability. The integrated platform leverages the colorimetric signal for rapid MPXV screening, while the fluorescence mode enables quantitative detection with a remarkably low detection limit of 0.0024 ng/mL. This dual-signal approach significantly expands the application range

of current ICA techniques, providing a powerful tool for infectious disease diagnostics. These methods enhance sensitivity, enable simultaneous detection of multiple pathogens, and provide precise, distinguishable results based on color changes. This innovative new approach holds great potential for improving diagnostic accuracy and efficiency in diverse applications, particularly in point-of-care settings.

Several studies have reported the incorporation of LFA combined with different types of nanomaterials [128]. Recently, Chen et al. proposed a ratiometric fluorescence LFA with melamine-coated gold nanoparticles to create crescent-shaped Janus nano assemblies [129]. These were applied to reduce signal interference by establishing a colorimetric and ratiometric fluorescence dual-mode lateral flow immunoassay. It is the first time we successfully established a colorimetric and ratiometric fluorescence dual-mode lateral flow immunoassay (Au-AIENPs-RLFIA) for the visual and quantitative detection of aflatoxin B1 (AFB1). Another work was introduced into the LFA system to replace common spherical SERS nanotags for bacteria detection [130]. The antibody-labeled tags can efficiently and tightly cover the bacteria surface as nano stickers, providing stronger SERS signals and facilitating the fluidity of bacteria–nanotag complexes, thereby improving the detection sensitivity and multiplex ability of LFA for *Salmonella typhimurium* (*S. typhi*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Listeria monocytogenes* (*L. mono*) within 20 min (Figure 4). Another work demonstrated an advanced lateral flow immunoassay platform with dual-functional [colorimetric and surface-enhanced Raman scattering (SERS)] to detect the spike 1 (S1) protein of SARS-CoV-2 [131]. The nanosensor was integrated with a specially designed core–gap–shell morphology consisting of a gold shell decorated with external nanospheres to produce a strong colorimetric signal and an enhanced SERS signal. This methodology showed excellent sensitivity, reproducibility, and rapid detection of the SARS-CoV-2 S1 protein, demonstrating excellent potential as a promising point-of-care platform for the early detection of respiratory virus infections. Wang and co-workers developed a multi-layered quantum dot-based silica nanoparticle marker combined with a fluorescent lateral flow assay (NFLFA) for nucleic acids, designed to identify *Acinetobacter baumannii* (CRAB) by targeting two genes: *RecA* (T1), a carrier gene, and *blaOXA-23* (T2), a drug-resistant gene [132]. To enable simultaneous and rapid quantitative detection of both amplified DNA types, loop-mediated isothermal amplification (LAMP) was integrated into the system. The fluorescent properties of quantum dots (QDs) enhanced the assay's accuracy in detecting the T1 and T2 analytes, achieving detection limits of 199 CFU/mL for *RecA* and 287 CFU/mL for *blaOXA-23*. This method allows rapid detection without requiring complex equipment or specialized training. Combining lateral flow assays with colorimetric techniques has offered a viable solution to meet these demands. LFA provides a simple, rapid, and user-friendly platform, while colorimetric detection enhances the visual interpretation of results without the need for complex instruments. This integration allows for the development of diagnostic devices that are not only efficient and cost-effective but also accessible for point-of-care testing. By utilizing the complementary strengths of LFA and colorimetric methods, it is possible to achieve accurate and timely diagnostics, particularly in resource-limited settings.

Another technique that demonstrates significant synergy is the combination of colorimetric methods with electrochemistry. Many nanomaterials exhibit notable electrochemical activity, which can be attributed to their intrinsic properties. For instance, gold electrodes, whether in nanoparticle form or not, can facilitate the formation of a self-assembled monolayer (SAM), enabling the detection of virus-derived proteins such as the recombinant nucleocapsid protein (rN) from SARS-CoV-2 [133]. Additionally, integrating electrochemistry with microfluidics can enhance the functionality of these electrodes, enabling the detection of IgG antibodies against *Toxocara canis* (IgG anti-*T. canis*) [134]. This combined

approach leverages the sensitivity of electrochemical detection and the precision of microfluidics, offering a powerful platform for accurate and efficient diagnostics. For the latter, an immunosensor was developed to diagnose toxocariasis. This disease represents a significant public health problem due to the limited available drugs of treatments and its association with neurodegenerative diseases. Another demonstration of synergy between these two techniques involves the detection of the SARS-CoV-2 spike antigen using gold nanoparticle-based biosensors [135]. In the presence of the SARS-CoV-2 spike antigen, gold nanoparticles aggregated rapidly and irreversibly due to the antibody-antigen interaction, resulting in a visible color change from red to purple. This colorimetric change was detectable with the naked eye or through UV-Vis spectrometry, exhibiting a spectral redshift and a detection limit of 48 ng/mL. Additionally, electrochemical detection was performed by applying the developed probe solution onto a commercially available, disposable screen-printed gold electrode, without the need for electrode preparation or modification. This method achieved a detection limit as low as 1 pg/mL for the SARS-CoV-2 spike antigen. Both colorimetric and electrochemical methods demonstrated high specificity for the SARS-CoV-2 spike antigen, effectively distinguishing it from other antigens, such as influenza A (H1N1), MERS-CoV, and *Streptococcus pneumoniae*, even at high concentrations.

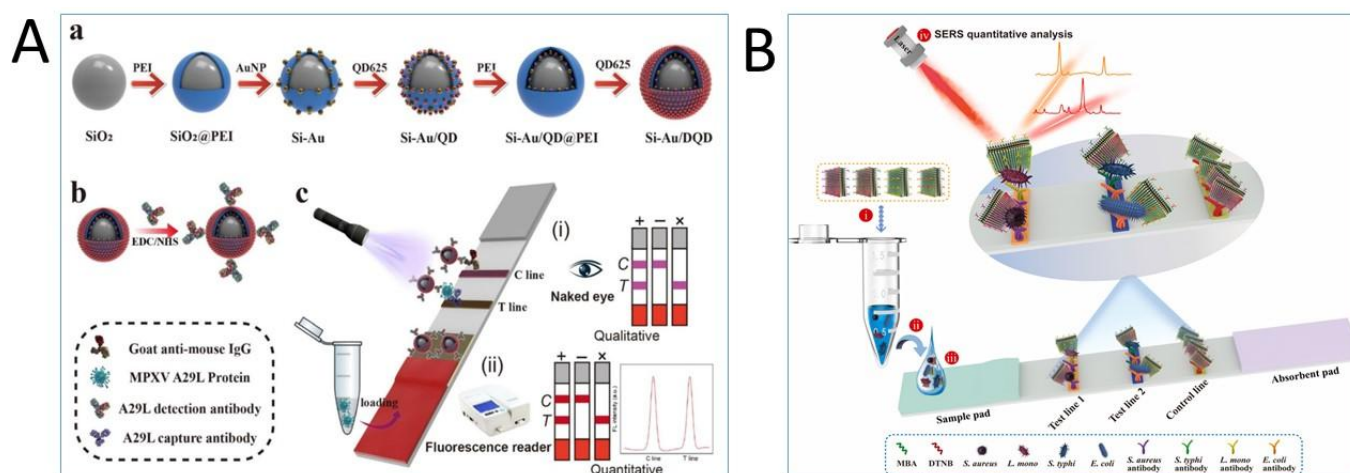


Figure 4. (A) (a) Schematic representation of the biosensor. (a) SiO₂-AuNPs nanocomposite synthesis. (b) MPXV A29L antibody coupling method, and (c) schematic diagram of SiO₂-AuNPs-based LFIA analysis of MPXV A29L protein, (i) naked eye and (ii) fluorescence reader. Reproduced with permission [116]. Copyright 2023, Springer Nature. (B) Schematic diagram of GO@Au-/Ag-based SERS-LFA mechanism for multiplex detection of four bacteria [131]. Copyright Elsevier.

6. Conclusions

As outlined in this review, various colorimetric strategies based on functionalized nanomaterials have been extensively utilized for pathogen detection, highlighting key characteristics such as functionalization, repeatability, and sensitivity, which are summarized in Table 1. Despite significant progress in constructing nanomaterial-based colorimetric systems, this promising field faces several challenges and limitations. We have discussed primary sensing strategies relevant to pathogen detection, many of which can produce rapid results within minutes, with sensitivities comparable to or exceeding those of conventional assays. But there is still much work to be conducted. Firstly, improving the selectivity and stability of colorimetric sensors is crucial for detecting pathogens in real-world samples. Decreasing the size of nanomaterials can increase the specific surface area, thereby enhancing sensitivity. A stable signal output pattern also reduces the likelihood of false positive results. Developing novel colorimetric sensors that address public health concerns, including emerging infectious diseases, should also be a priority. Secondly, current

colorimetric systems are often limited to detecting a single type of pathogen and struggle with simultaneously detecting multiple pathogens. While machine-learning-assisted sensor arrays offer promise in identifying pathogen species, quantitative analysis of concentrations remains underexplored. Enhancing the performance of colorimetric sensors for various analyte detection is vital for achieving both qualitative and quantitative analysis of multiple targets. Integrating advanced machine learning techniques with array-based sensors, such as convolutional neural networks, could enable more accurate pathogen analysis. Combining colorimetric systems with multichannel microfluidic systems presents a promising approach to improving multi-target detection capabilities. Recent advancements have focused on fabricating enzyme-like functional nanomaterials and 3D-structured lateral flow assays for pathogen detection. Unlike traditional aggregation-based methods primarily used for signal visualization, these methodologies are less affected by the complex components of real samples, providing more accurate signals. These advanced sensing systems can quantitatively detect bacteria and viruses across a broad range using digital cameras, smartphones, or mobile devices to capture color intensity and process data. Lateral flow formats offer a user-friendly, transportable assay method and, when combined with sensitive electrochemical or fluorescent signal transduction, can become viable alternatives to RT-PCR and ELISA due to their quantitative sensitivity. In conclusion, while the field of nanomaterial-based colorimetric sensors has made notable strides, there remains significant potential for innovation and improvement. Addressing challenges such as multi-target detection, signal stability, and integration with artificial intelligence will be critical for advancing these systems into more robust, practical tools for pathogen detection in diverse settings.

Table 1. Applications of colorimetric sensors for pathogen detection.

Type of Pathogens	Analyte	Strategy	LOD	Ref.
<i>Staphylococcus aureus</i>	Sugar cane	Antibody/Aggregation	105 CFU/mL	[37]
Dengue	Serum	Antibody/Aggregation	TCID ₅₀ 10 ⁷	[38]
PCV-2	Serum	Antibody/Aggregation	10 ⁵ DNA copies/mL	[40]
<i>Staphylococcus aureus</i>	Milk	Antibody/Magnetic/Aggregation	1.5 × 10 ⁵ CFU/mL	[43]
Influenza virus A	Blood sample	Peroxidase mimic	1.11 pg/mL	[48]
<i>Ersinia enterocolitica</i>	Human serum	Enzyme mimics	30 CFU/mL	[49]
Methicillin-resistant <i>Staphylococcus aureus</i>	Food sample	Aptamer/Aggregation	20 nM	[52]
<i>E.Coli</i>	Food sample	fluorescein-labeled aptamer	10 CFU/mL	[58]
Human adenovirus	Human sample	Antibody/Aggregation	10 ⁴ copies/mL	[60]
Influenza A and B viruses	Human sample	Antibody/Aggregation	0.04 ng mL ⁻¹	[62]
<i>E. coli</i> O157 <i>S. typhimurium</i> <i>V. parahaemolyticus</i> .	Food sample	Aptamer/Aggregation	39 CFU/mL 60 CFU/mL 60 CFU/mL	[65]
H5N1 virus	Human sample	Aptamer/Aggregation	11.6 fM	[66]
<i>Listeria monocytogenes</i>	Food samples	Aptamer/Aggregation	2.83 × 10 ⁵ CFU/mL	[70]
<i>E. coli</i> <i>Klebsiella pneumoniae</i>	Urine samples	Catalyze H ₂ O ₂	512 × 10 ⁵ CFU/mL	[77]
Ochratoxin A	Food samples	Antibody/Aggregation	0.001 pg mL ⁻¹	[79]

Table 1. Cont.

Type of Pathogens	Analyte	Strategy	LOD	Ref.
<i>E. coli</i> <i>Staphylococcus aureus</i>	Minced chicken	Antibody / Aggregation	50 CFU/mL	[80]
<i>Aggregatibacter actinomycetemcomitans</i> <i>Actinomyces naeslundii</i> <i>Porphyromonas gingivalis</i> <i>Streptococcus oralis</i>	Human sample	Positively/Negatively charged gold nanoparticles	10 ⁷ CFU/mL	[81]
<i>Staphylococcus aureus</i>	Milk and infant food	Aptamer / Aggregation	7.5 × 10 ⁴ CFU/mL 8.4 × 10 ⁴ CFU/mL	[82]
<i>Mycobacterium</i>	Clinic samples	Antibody / Aggregation	0.0625 ng/mL	[83]
<i>Escherichia coli</i> <i>Salmonella</i>	Food samples	Antibody / Aggregation	10 ³ CFU/mL 10 ² CFU/mL	[84]
<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	Clinic samples	Protein / Aggregation	10 ⁵ CFU/mL 10 ⁸ CFU/mL	[85]
<i>Zika virus</i>	Human serum	Antibody / Aggregation	0.96 µg mL ⁻¹	[88]
<i>Corona virus</i>	Saliva	Enzyme / Aggregation	625 PFU/mL	[89]
<i>Klebsiella pneumoniae</i>	Urine	Aptamer / Aggregation	3.4 × 10 ³ CFU/mL	[93]
<i>Vibrio cholerae</i>	Human sample	Aptamer / Aggregation	10 ³ CFU/mL	[94]

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