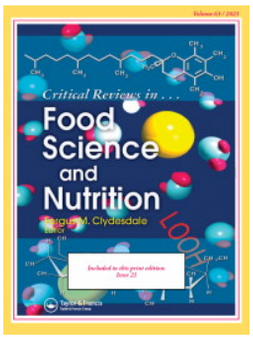

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REVIEW ARTICLE

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A review of rapid food safety testing: using lateral flow assay platform to detect foodborne pathogens

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ABSTRACT

The detrimental impact of foodborne pathogens on human health makes food safety a major concern at all levels of production. Conventional methods to detect foodborne pathogens, such as live culture, high-performance liquid chromatography, and molecular techniques, are relatively tedious, time-consuming, laborious, and expensive, which hinders their use for on-site applications. Recurrent outbreaks of foodborne illness have heightened the demand for rapid and simple technologies for detection of foodborne pathogens. Recently, Lateral flow assays (LFA) have drawn attention because of their ability to detect pathogens rapidly, cheaply, and on-site. Here, we reviewed the latest developments in LFAs to detect various foodborne pathogens in food samples, giving special attention to how reporters and labels have improved LFA performance. We also discussed different approaches to improve LFA sensitivity and specificity. Most importantly, due to the lack of studies on LFAs for the detection of viral foodborne pathogens in food samples, we summarized our recent research on developing LFAs for the detection of viral foodborne pathogens. Finally, we highlighted the main challenges for further development of LFA platforms. In summary, with continuing improvements, LFAs may soon offer excellent performance at point-of-care that is competitive with laboratory techniques while retaining a rapid format.

KEYWORDS

lateral flow assay; LFA; food safety; foodborne pathogens; sensitivity

Introduction

In the last decade, outbreaks of foodborne diseases from various food sources have raised public awareness of food safety (Karp et al. 2015). According to the World Health Organization (WHO 2022), around 600 million individuals – almost 1 in 10 people worldwide– acquire foodborne infections after eating contaminated food each year. In addition, nearly 420,000 individuals die yearly from diarrheal disorders (WHO 2022). A substantial number of these fatalities were avoidable through early detection of pathogens in food and water (WHO 2022, 2016). Unfortunately, children under five years of age carry 40% of the foodborne disease burden, with 125,000 deaths yearly (WHO 2022). The symptoms of foodborne diseases range from simple gastroenteritis to potentially catastrophic neurologic, hepatic, and renal complications (Fung, Wang, and Menon 2018). The majority of foodborne diseases are attributed to bacteria (*Campylobacter spp., Salmonella spp., Staphylococcus aureus (S. aureus), Vibrio cholera (V. cholera)*, *Escherichia coli (E.coli) O157:H7*, *Clostridium perfringens*, and *Listeria monocytogenes (L. monocytogenes))*, viruses (Norovirus, Hepatitis E, Hepatitis A, Rotavirus, Adenoviruses, Sapoviruses, and Astroviruses), and protozoa (*Cryptosporidium* spp., *Cyclospora spp.*, and *Toxoplasma* spp.) (Bintsis 2017; Adley and Ryan 2016).

Foodborne diseases impede socioeconomic development by straining healthcare systems and harming national economies, tourism, and international food trade. Around $110 billion is lost annually in productivity and medical expensesas a result of contaminated food with foodborne pathogens in low-income and middle-income countries (WHO 2022, 2016). In addition, globalization of trade has increased the risk of the transnational spread of foodborne diseases in the current scenario. Although they were once limited to

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small communities, many outbreaks of foodborne diseases now have global consequences (Scott 2003).

Implementing effective monitoring systems that include laboratory readiness is one of the most rational and reasonable strategies to avoid or minimize the harmful effects of foodborne diseases in people. Diagnostic food labs play critical roles in identifying and isolating foodborne pathogens using conventional and molecular diagnostic methods, which are the cornerstone of pathogen detection and identification. However, these methods are relatively tedious, time-consuming, laborious, and expensive. Therefore, on-site quick diagnostic techniques that are robust, efficient, sensitive, and cost-effective are urgently needed to speed up the detection of foodborne pathogens.

In the past 25 years, biosensors for rapid pathogen detection have been developed based on integrating a sensitive transducer and a selective biorecognition element. These biosensors provide quantitative or semiquantitative analytical measurements without requiring other chemicals or processing steps (Cesewski and Johnson 2020), enabling on-site pathogen quantification and identification that complement laboratory-based methods like polymerase chain reaction (PCR) and Enzyme-linked immunosorbent assay (ELISA) (Sohrabi et al. 2021). Biosensors have been implemented in various analytical techniques for environmental, medical, food safety, industrial processing, defence, and security applications (Arora, Chand, and Malhotra 2006). During the COVID-19 pandemic, lateral flow assays (LFAs) attracted wide attention as one of the most important biosensing platforms; however, extensive efforts are still being made in academia and industry to improve the performance of LFA-based testing (Kim and Lee 2022).

In this review, we highlighted the principles and features of LFA-based strategies for foodborne pathogens detection in food samples, focusing on recent improvements in LFA platforms for ultra-sensitive detection of foodborne pathogens. We also discussed different approaches to improve LFA sensitivity and specificity. We also address the utilization of various reporters for signal amplification, including; nanoparticles (NPs) (Pashazadeh-Panahi et al. 2021), nanomaterials (Soozanipour et al. 2021) and other labeling materials. Most importantly, due to the lack of studies on LFA for the detection of viral foodborne pathogens in food samples, we summarized our recent research on developing LFA for the detection of viral foodborne pathogens. In summary, with continuing improvements, LFAs may become the fastest (<30 min), ultrasensitive (PCR-level), and "sample-to-answer" point of care (POC) diagnostics test.

Current challenges in conventional methods for foodborne testing

Current technologies for foodborne pathogen screening require labor-intensive sample enrichment steps, pathogen

Table 1. Five main types of technologies used to identify foodborne pathogens.

Technology	Laboratory-Based or On-Site	Time to Results	Cost $	Specificity (%)	Number of Samples/Batches	Special instrument required	Viral and parasatic foodborne pathogen detection	Limitations	Ref
Culture	Laboratory-Based	Minimum 2-3 days up to a week	3-6	100	1 sample	Yes	No	Time-consuming, laborious, and must be followed by biochemical tests ('metabolic fingerprinting'), molecular tests (typically PCR), or mass spectrometry. Needs at least a BSL2 laboratory.	(Bouguelia et al. 2013)
PCR	Laboratory-Based	~ 4 h	20	100	Up to 96 samples	Yes	Yes	Expensive, equipment, laborious, time-consuming, and highly trained personnel	(Liu, Cao, et al. 2019)
LAMP	On-Site	2–3 h	10-20	100	1-4 samples	Yes	Yes	High rate of false positivity due to heavy dependence on indirect detection methods like turbidity and nonspecific dye	(Buddolla and Kim 2021)
ELISA	Laboratory-Based	~4-6 h	10	70-90	Up to 96 samples	Yes	Yes	Low sensitivity	(Gomaa and Boye 2015) (Török et al. 2015)
LFA	On-Site	15–20 min	1	100	1 sample	No	Yes	Low sensitivity and specificity	(Zhao et al. 2016a) (Tominaga and Ishii 2020)

For sensitivities (%), Please refer to the text in section 2, as it varies significantly depending on the foodborne pathogen.

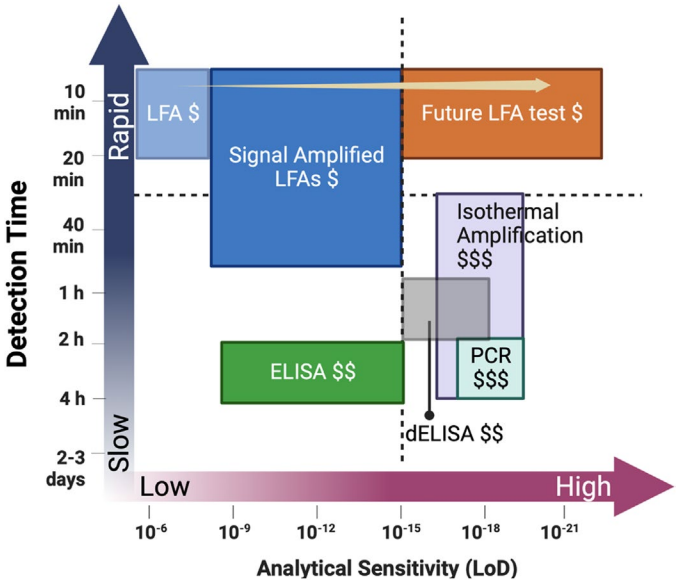

Figure 1. Comparison of the sensitivity, price ($least expensive; $$more expensive; $$$most expensive), and detection time of signal-amplified LFAs compared emerging isothermal nucleic acid amplification diagnostics, PCR, digital enzyme- linked immunosorbent assay (dELISA), and commercial diagnostic tools. Figure created using BioRender.com and adapted from (Liu et al. 2021).

isolation and purification, and costly readout machinery. Table 1 **and** Figure 1 summarize the advantages and disadvantages of the 5 main types of technologies used to identify foodborne pathogens in food samples.

Conventional live culture remains the gold standard for foodborne pathogen detection and identification; however, this method is tedious, laborious, and time-consuming (2–3 days) (Zhao et al. 2014). Culture methods have also been reported to show poor sensitivity for low-level contamination with a high background of indigenous microflora in the samples, rendering the recovery of the target organism difficult. Although it is highly specific, conventional culture method sensitivities vary depending on the type of pathogen. For example, the limit of detection (LoD) for the detection of; *Salmonella Spp* was 1.76 colony forming unites/ ml (CFU/ml) (Sharif and Tayeb 2021), for *Campylobacter spp* was $0.3–5\times10^6$ CFU/mL (Buss et al. 2019), for *Shigella spp* was 10^6 CFU/mL (Jiménez, McCoy, and Achí 2010), for *Listeria spp* was $\leq10^4$ cells/mL (Gasanov, Hughes, and Hansbro 2005), and for *Escherichia coli* (E. coli) was 10^2 CFU/g (J. O'Sullivan et al. 2007). Most importantly, false negative results may occur due to viable but non-culturable pathogens. The failure to detect foodborne pathogens would increase the transmission risk of pathogens.

One of the most commonly used molecular-based methods for the detection of foodborne bacterial pathogens is PCR. PCR is effective clinical procedure for the rapid detection and recognition of pathogens in the healthcare system (Kawasaki et al. 2009, 2010); however, they are also expensive, requires specialized equipment and highly trained personnel, and relies on extensive sample pretreatment and costly instruments (Buckwalter et al. 2014). PCR have been used in the detection of numerous foodborne pathogens like *Salmonella spp* (LoD:10^3 CFU/mL (Tang et al. 2018)), *Campylobacter jejuni* (LoD: 10^2 CFU/ml (Jelenik et al. 2005)), *Shigella spp* (LoD: 10^1 CFU/mL (Tang et al. 2018)), *Listeria spp* (LoD:10^3–10^4 CFU/mL (Li, Ye, et al. 2020)), and *E. coli O157:H7* (LoD: 10^3 CFU/mL (Wei et al. 2018)).

Loop-mediated isothermal amplification (LAMP), has been regarded as an innovative gene amplification technology and emerged as an alternative to PCR-based methodologies in both clinical laboratory and food safety testing. Due to its rapidity and sensitivity, LAMP has been used to detect various foodborne pathogens. LAMP is proven to be more specific and sensitive as compared to PCR assays for the detection of foodborne pathogens including; *Vibrio parahaemolyticus (V. parahaemolyticus)* (LoD:10 CFU/reaction (Wang et al. 2013)), *Vibro vulnificus* (LoD: 2.5x 10^3CFU/g (Han, Wang, and Ge 2011)), *Salmonella* (LoD: 10^1 CFU/mL (Techathuvanan, Draughon, and D'Souza 2010)), *S. aureus* (LoD: 3.4 CFU/g (Jiang et al. 2020)), and *Shigella* (LoD: 5CFU/10 mL). Commercial LAMP kits are available for the detection of Enterohemorrhagic Escherichia Coli (*EHEC)*, *Salmonella* and *L. monocytogenes* (Yamazaki et al. 2018)

Antibody-based immunoassays such as ELISA (Shen et al. 2014) are easier to perform than other antibody-based methods, but it is still difficult to deploy in on-site settings because of its requirements for special equipment and operating expertise (Zhao et al. 2014). In addition, the relatively poor sensitivity of ELISA remains a significant drawback. Many studies have been performed using ELISA for rapid detection of foodborne pathogens such as *Salmonella* (LoD: 10^4 to 10^5 CFU/mL (Paniel and Noguer 2019)), *Campylobacter* (LoD: 10^5 to 10^6 CFU/mL (Hochel et al. 2007), *Listeria* (LoD: 6.6×10^3 CFU/mL (Portanti et al. 2011)), *E.coli* (LoD: 6.8×10^2 to 6.8×10^3 CFU/mL (Shen et al. 2014)), and *V. parahaemolyticus* (LoD: 10^3 cells (Kumar et al. 2011)). Commercial ELISA test kit such as BIOLINE *Salmonella* ELISA is also available to detect *Salmonella* in food products. The LoD of this test kit was 1 CFU/25 g sample with a minimum of four of the 20 food matrixes tested (Bolton et al. 2000). In addition, high-throughput and automated ELISA systems such as VIDAS (BioMerieux) and Assurance EIA (BioControl) are available for the detection of foodborne pathogens (Glynn et al. 2006). Several studies applied VIDAS for the detection of *Salmonella* in pork samples, fruits and vegetables (Vieira-Pinto et al. 2007; Gomez-Govea et al. 2012), *L. monocytogenes* in fish samples, beef, pork, fruits, and vegetables (Vaz-Velho, Duarte, and Gibbs 2000; Meyer et al. 2011; Gomez-Govea et al. 2012), *E. coli* O157:H7 in Minas Frescal cheese, fruits, and vegetables (Gomez-Govea et al. 2012; Carvalho et al. 2014), *Campylobacter* spp. in fruits and vegetables (Gomez-Govea et al. 2012) and staphylococcal enterotoxin in raw milk cheese (Cremonesi et al. 2007).

The recent progress in multi-gene detection technology includes microarray technology (Call, Brockman, and Chandler 2001). Microarrays were initially used for the study of gene expression, but oligonucleotide DNA microarray has been widely studied for the detection of foodborne pathogens. Wang et al. developed a microarray assay that detected and identified 22 foodborne pathogens (Wang et al. 2007)

including; *S. aureus, L. monocytogenes*, *V. parahaemolyticus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Clostridium perfringens*, *Shigella* spp. *Salmonella* spp., and *Bacillus cereus (B. cereus)* with LoD varying between 10^1 to 10^3 CFU/ml (Wang et al. 2007). Despite their high sensitivity, microarrays are not desirable for microbial food analysis because low to medium density array will serve as the ideal microarray platform that can provide reliable results without involving the use of complicated equipment's and data management. The most significant disadvantages of microarrays include the low accuracy, precision, specificity, and high cost of a single experiment.

Chromatographical methods are another standard technique for food testing (Eugster et al. 2011). They are sensitive and accurate despite their many drawbacks, such as being time-consuming, tedious, laborious, multi-complex, and limited for detecting bacterial-borne pathogens. Sun et al. (Sun et al. 2017) developed a multiplex PCR-based procedure followed by high-performance liquid chromatography (mPCR-HPLC) assay for high-throughput screening foodborne pathogens, including; *Salmonella spp., L. monocytogenes, Enterobacter sakazakii, S. aureus, Shigella spp., E. coli O157:H7, V. parahaemolyticus, Vibrio cholerae, and Vibrio vulnificus*. The detection limit of mPCR-HPLC was 10^1 CFU/mL in pure cultures and less than 10^2 CFU/g in contaminated matrixes (Sun et al. 2017).

None of those mentioned above methods perfectly fulfill the criteria for the urgently required on-site multiplex detection system for foodborne pathogens. Recently, LFA has evolved to fill this gap and to offer performance at the POC that is competitive with laboratory techniques while retaining a rapid format. In the following sections, the principles and features of LFA-based strategies for foodborne pathogens detection in food samples were highlighted, focusing on recent improvements in LFA platforms for detecting bacterial and viral foodborne pathogens.

LFA: definition and assay formats

The LFA is a paper-based platform for detection and quantification of analytes in complex mixtures, where the sample is placed on a test device, and the results are displayed within 5–30 min. Atypical LFA has four components: a sample pad, a conjugate release pad, a nitrocellulose membrane (NC), and an absorption pad. All four components are laminated onto a sheet of plastic backing. Sandwich and competitive assays are the two standard formats for LFA, and each has distinct characteristics and benefits.

Sandwich LFA

In the sandwich assay format, three different antibodies are usually used; (1) conjugate antibodies, immobilized on the conjugation pad, which recognize one epitope in the target. These antibodies are linked to reporter particles, (2) capture antibodies, immobilized at the test line on the NC, which recognize another epitope of the target, and (3) anti-species antibodies, which are immobilized on the control line. Positive results are obtained when the conjugated labeled antibody (Ab) antigen (Ag) complex binds to the antibodies on the test line, and any extra labeled antibodies are collected at the control line forming two lines as shown in Figure 2. Negative results are obtained when the reaction antibody only reacts with the anti-species antibodies on the control line. Hence,

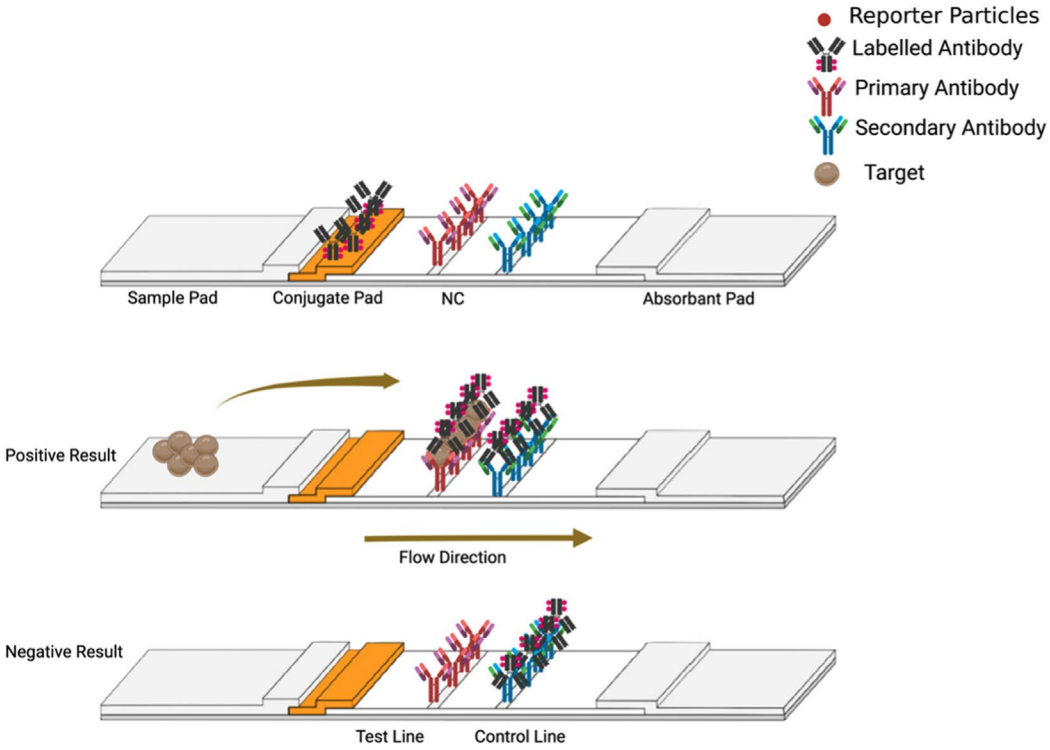

Figure 2. Schematic design of a sandwich LFA. When the analyte binds to both the capture antibody immobilized at the test line and the detection antibody, a colour develops at the test line, indicating a positive result. Figure created using BioRender.com. NC: Nitrocellulose membrane.

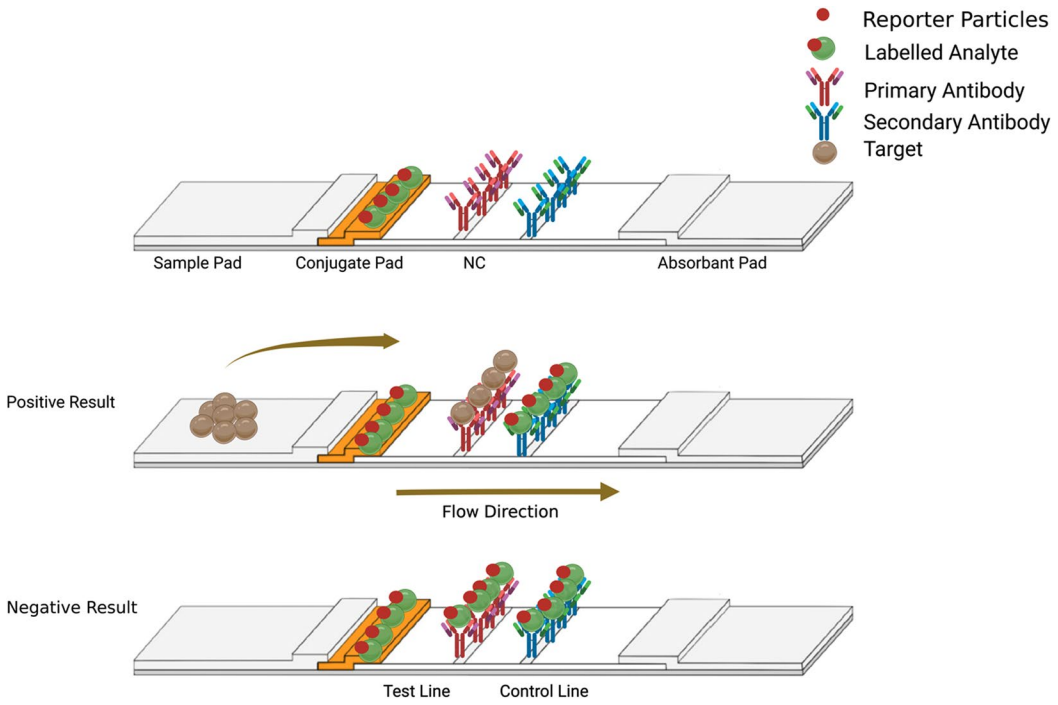

Figure 3. Schematic diagram shows the competitive format (First setup). The color intensity developed at the test line is inversely proportional to the amount of analyte. Figure created using BioRender.com.

only one red line will develop at the control line. The quantity of analyte present in the sample can be determined by the color intensity that is visible at the test line. Typically, this test format is used for large analytes with multiple epitopes.

Competitive LFA

There are two different setups that may be used in the competitive immunoassay format. In the first setup (Figure 3), the labeled analyte is immobilized on the conjugation pad. When the target is absent in the sample, the labeled analyte flows and binds to the detection antibody and the secondary antibody (anti-species) immobilized on the test and control line, respectively (Sajid, Kawde, and Daud 2015). Hence, two red lines develop on the test and control lines. When the target analyte is present in the sample, the unlabeled analyte competes with the labeled analyte immobilized on the conjugation pad and binds to the test line. Whereas the labeled analyte binds to the secondary antibodies on the control line. Hence, only a single red line develops on the control line (Pohanka 2021).

In the second setup (Figure 4), the labeled antibodies are immobilized on the conjugation pad. When the target is absent in the sample, the labeled antibodies flow and bind to the target analyte carrier and the secondary antibody on the test and control line. Hence, two red lines develop. When the target analyte is present in the sample, the target analyte binds to the labeled antibodies immobilized on the conjugation pad. Hence, these labeled antibodies will not be able to bind to the target analyte carrier on the test lines. However, the labeled antibodies conjugated to the target analyte will bind to the secondary antibodies immobilized on the control line. Thus, only a single red line develops on the control line.

Reporter agents and reading instruments

An optical signal is generated from reporter particles bound at the test line in the LFA. This signal can be read qualitatively or semi-quantitatively by the naked eye or using an optical reader. To maximize the sensitivity of an LFA, each binding event between the target and the reporter should produce the strongest possible signal. Large reporters usually result in robust signal per binding event; however, reporter particles that are too large do not easily flow through the NC and have fewer opportunities to bind at the test line. Therefore, a small reporter with a diameter ranging between 20 to 500 nm is typically selected for use in LFA.

In recent years, the utilization of novel nanomaterials as reporter molecules has increased dramatically. Gold nanoparticles (AuNPs) are the most commonly used reporters in mass-produced tests (Nguyen et al. 2020; Ge et al. 2014; Quesada-González and Merkoçi 2018), as they permit naked-eye detection. This is highly advantageous for quality applications or applications seeking cost efficiency, as it does not require an external reader. Combining nanoparticle-based detection with an external reader may increase reproducibility and provide quantitative results. The following sections highlight what we consider to be the most significant nanoparticles in terms of their readout type (see Table 2 for a summary of commercially available reporter agents).

Gold nanoparticles (AuNPs)

Since the 1980s, AuNPs have been the most frequently used detection labels in LFAs (Verheijen et al. 2000; Fong et al. 2000; Shyu et al. 2002). The reasons for their popularity include (1) production of a robust red color for naked-eye

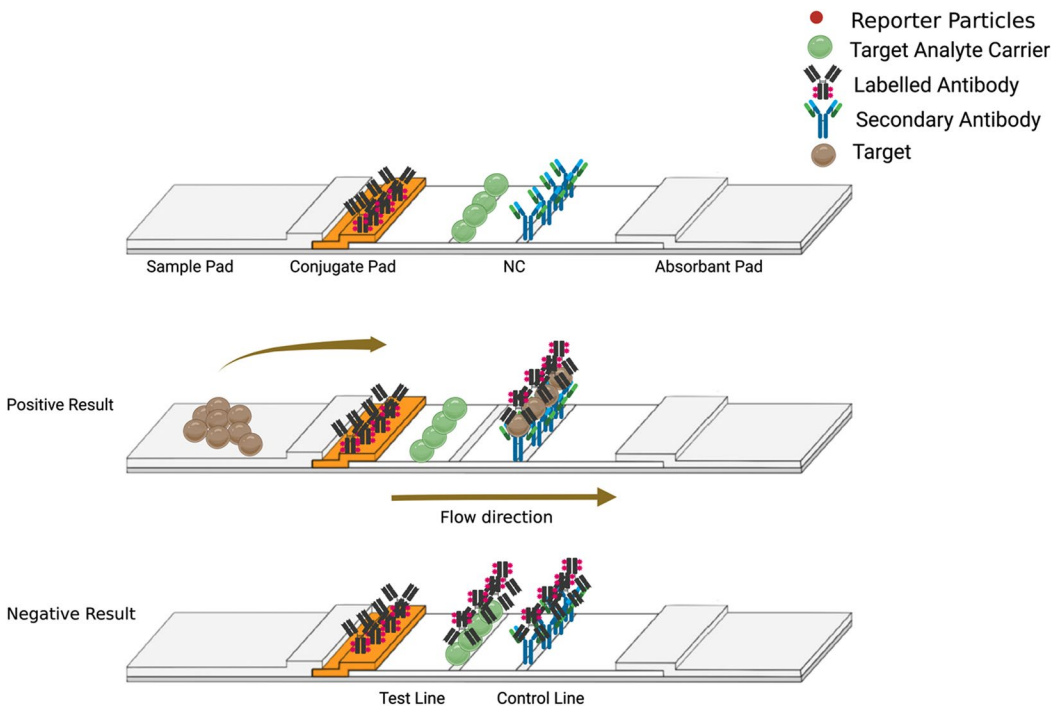

Figure 4. Schematic diagram shows the competitive format (Second setup). The color intensity developed at the test line is inversely proportional to the amount of analyte. Figure 4 created using BioRender.com.

Table 2. Advantages and disadvantages of commercially available reporter agents.

Label	Advantages	Disadvantages
AuNPs	• Qualitative naked-eye detection • Easy conjugation protocol • Strong signal • Quantitative detection by external readers (became very affordable)	• Low sensitivity
Carbon nanoparticles/ carbon nanotubes	• Qualitative naked-eye detection • High signal-to-noise ratio • Cheaper than AuNPs • Very Stable	• External reader needed for quantitative detection • Nonspecific adsorption • Weaker signal than AuNPs
Latex beads	• Qualitative naked-eye detection • Resistant to chemical and physical damage • Cheaper than AuNPs • Multiple colors	• External reader needed for quantitative detection • Less sensitive • Weaker signal than AuNPs
QDs	• Strong signal • Multiple colors	• UV external light reader needed for quantitative detection • Higher toxicity
UCNPs	• Strong fluorescent signal • Safer than QDs, as they do not require a UV source • Multiple colors	• Near-infrared laser needed for quantitative detection • More expensive than QDs
Liposomes	• Multiplexing • Easy conjugation	• Require extra hardware depending on the loaded label • Sensitive to pH and ionic strength
Magnetic nanoparticles	• Dual magnetic/colourimetric signal • High signal-to-noise ratio • Very sensitive	• Require a non-optical reader for magnetic measurements

detection, (2) availability in different sizes and shapes, (3) low toxicity, (4) ease of functionalization *via* covalent bonding (Parolo, de la Escosura-Muñiz, and Merkoçi 2013; Di Nardo et al. 2019; Mao et al. 2009), and (5) high stability. The size and shape of conventional AuNPs can also be modified to achieve higher sensitivity. In addition, the optical signal of AuNPs in colorimetric LFAs can be amplified by depositing enzymes and silver ions (Sajid, Kawde, and Daud 2015). AuNPs produce red bands at the test and control lines of the LFA when acting as reporter particles. AuNPs can also act as carriers if coupled with an antibody modified with horseradish peroxidase (HRP) enzyme. Once substrates are added, they produce insoluble chromogens, which cannot be moved by the flow, concentrating the color at the test and control lines. LFAs that utilize the HRP enzyme produce two different optical signals: one produced by the red color of the AuNPs and the other by the substrate of the HRP, which is more sensitive, achieving an 'on-demand' tuning of the biosensing performance (Parolo, de la Escosura-Muñiz, and Merkoçi 2013).

Another method to enhance the sensitivity of AuNPs is by using silver ions, which tend to gather around the

Table 3. Summary of studies on LFAs for the detection of foodborne pathogens.

Probe	Receptor	Sample	Target foodbourne pathogen	Qualitative/Quantitative	Assay time	LOD	Refs
AuNP	Antibody	Chicken, raw pork, eggs, milk, cheese, raw shrimp, fish, codfish, broccoli, and fruit juice.	*V. parahaemolyticus; S. aureus; Salmonella enteritidis (S. enteritidis); E. coli O157:H7; L. monocytogenes*	Qualitative: Naked eye Quantitative: test strip reader	15 min	10^1 CFU/mL	(Jin et al. 2022)
Up-converting phosphor (UCP)	Antibody	Food and water.	*E. coli O157:H7; V. cholera O1; V. cholera O139; Salmonella paratyphi (S. Paratyphi) A; S. paratyphi B; S. paratyphi C; S. enteritidis; Salmonella Typhi (S. typh)i;V. parahaemolyticus*	Quantitative: TC-UPT-LF biosensor	20 min	10^4 CFU/mL - 10^5 CFU/mL	(Zhao et al. 2016b)
AuNPs + HRP-TMB	Antibody	Buffer	*S. typhi*	Quantitative: colourimetric signals in captured images were digitized using house-made image analysis software.	16 min	9.2 x 10^3 CFU/ml	(Park, Kim, and Kim 2010)
AuNPs	Antibody	Milk	*S. typhi*	Qualitative: Naked Eye	30 min	10^2 cells	(Pandey et al. 2012)
AuNPs	Antibody	Water	*S. typhi*	Qualitative: Naked Eye	10 min	1 x 10^4 CFU/ml	(Singh, Sharma, and Nara 2015b)
AuNPs	Antibody	Human serum	*S. typhi*	Qualitative: Naked Eye	15 min	1.14 x 10^5 CFU/rnl	(Preechakasedkit et al. 2012)
AuNPs	DNA	Milk, juice, egg, wastewater	*S. typhi*	Qualitative: Naked Eye Quantitative: The images of the test line of LFA were captured by an iPhone 6S, and the optical densities of the test strips were measured by the Image-Pro Plus 6.0 software.	1 h	1 x 10^3 CFU/ml	(Tang et al. 2017)
AuNPs + HAuC14	Antibody	Milk	*S. enteritidis*	Qualitative: Naked Eye	6 h	1 x 10^4 CFU/ml	(Bu et al. 2018)
AuNPs + magnetic nanoparticles (MNPs)	DNA Aptamer	Buffer	*S. enteritidis*	Qualitative: Naked Eye Semi-quantitative: Strip Reader	NA	1 x 10^1 CFU/ml	(Fang et al. 2014)
AuNPs	Antibody	Buffer	*S. typhi; S. enteritidis;*	Qualitative: Naked Eye	15 min	1 x 10^4 CFU/ml-1 x10^6 CFU/ml	(Moongkarndi, Rodpai, and Kanarat 2011)
Fe304/Au core/shell	Antibody	Milk	*S. enteritidis*	Qualitative: Naked eye and CG-ICTS reader	22 h	5 x IO^5 CFU/ml	(Xia et al. 2016)
Quantum dots (QDS) + MNPs	DNA	Raw meat milk	*S. aureus*	Qualitative: UV Light.	2 h	3 x 10^0 CFU/ml – 3×10 ^{1} CFU/ml	((Chen et al. 2014)
AuNPs	DNA	Pork	*S. aureus*	Qualitative: Naked Eye	48 h	2 x 10^2 CFU/g	(Zhang et al. 2017)
AuNPs + Silver	DNA	Buffer	*Salmonella spp,*	Qualitative: Naked Eye Quantitative: Quantity One (Bio-Rad)	40 min	10^4 cells/ml	(Liu et al. 2013a)
AuNPs	DNA	Buffer	*S. typhi*	Qualitative: Naked Eye Qualitative: ImageJ software	80 min	50 CFO	(Park et al. 2017)
AuNPs	DNA	Fecaluria	*S. typhi*	Qualitative: Naked Eye Quantitative: Quantity One (Bio-Rad)	30 min	5 x 10^8 CFU/ml	(Yeung et al. 2014)
AuNPs + MNPs	Antibody	Meat products, fish	*L. monocytogenes*	Qualitative: Naked Eye	15 h	1 x 10^2 CFU/10g	(Shim et al. 2008)
AuNPs	Antibody	Milk	*L. monocytogenes*	Qualitative: Naked Eye	13 h	3.7 x 10^6 CFU/ml	(Wang et al. 2017)
MNPs + HRP-TMB	Antibody	Buffer milk	*L. monocytogenes*	Quantitative: signal read by a scanner and the optical densities of the images were digitized	< 1 h	1 x 10^2 CFU/ml	(Cho and Irudayaraj 2013)
CNPs	DNA	Milk	*Listeria spp.; L. monocytogenes;*	Qualitative: Naked Eye	28 h	10^5 cells	(Blažková et al. 2009)
MNPs	Antibody	Food samples	*L. monocytogenes*	Qualitative: Naked Eye Qualitative: magnetic assay reader	25 min	1 x 10^4 CFU/ml	(Shi et al. 2015)
AuNPs/Ag core/shell	DNA	Milk, breast, beef, chicken	*L. monocytogenes; Staphylococcus epidermidis (S epidermidis)*	Qualitative: Naked Eye Quantitative: Raman detection reader	30 min	2 x 10^1 CFU/ml	(Liu et al. 2017)
QDs	Antibody	Buffer	*V. cholera*	Qualitative using UV lamp	10 min	1 x 10^3 CFU/ml	(Gharaat et al. 2017)
AuNPs	Antibody	Seafood	*V. cholerae 0139*	Qualitative: Naked Eye	20 min	1 x 10^4 CFU/ml	(Pengsuk et al. 2013)
MNPs	Antibody	Shrimp, clam	*V. parahaemolyticus*	Qualitative: Naked Eye Qualitative: Magnetic Assay Reader	NA	1 x IO^5 CFU/ml	(Yan et al. 2014)

(*Continued*)

Table 3. Continued.

Probe	Receptor	Sample	Target foodbourne pathogen	Qualitative/Quantitative	Assay time	LOD	Refs
MNPs	Antibody	Seafood	*V. parahaemolyticus*	Qualitative: Naked Eye Quantitative: Magnetic Assay Reader	4 h	1.58 x 10^2 CFU/ml	(Liu et al. 2015)
AuNPs	Antibody	Bread, milk, jelly	*E. coli 0157:H7; Shigella boydii;*	Semi-qualitative: Naked Eye	10 min	1 x 10^6 CFU/ml	(Song, Liu, Wu, et al. 2016)
AuNPs	Antibody	Lettuce	*E. coli 0157:H7; S. typhimurium*	Qualitative: Naked Eye Quantitative: signal quantification from images	20 min	1.87 x 10^4 CFIJ/rnl	(Shin et al. 2018)
AuNPs	DNA	Water, milk, blood, spinach	*E. coli; S. aureus*	Qualitative: Naked Eye Quantitative: Smart Phones	1 h	10-1000 CFU/ml	(Choi et al. 2016)
AuNPs + MNPs	DNA	Buffer	*E. coli; S. enteritidis*	Qualitative: Naked Eye Qualitative: FastGene FAST Digital System and the resulting images were processed with ImageJ software	NA	0.59-0.75 pg/ µL	(Ben Aissa et al. 2017)
Eu (III)-doped polystyrene nanoparticle (EuNPs)	Antibody	Milk	*E. coli 0157:H7*	Quantitative: portable strip reader for the EuNP-immunochromatographic test strip (ICTS) (excitation wavelength 1⁄4 365 nm, emission wavelength 1⁄4 615 nm)	30 min	1 x 10^2 CFU/ml	(Xing et al. 2018)
Fluorescein isothiocyanate (FITC)	Antibody	Bread, milk, jelly	*E. coli 0157:H7*	Qualitative or semi-quantitative: ESE-Quant Lateral Flow Reader	10 h	1 x 10^0 CFU/ml	(Song, Liu, Li, et al. 2016)
Concave palladium-platinum (Pd-Pt) nanoparticles as a nanozyme	Antibody	Milk	*E. coli O157:H7*	Qualitative: Naked Eye Quantitative: test strip reader	10 min	9×10^2 cfu/ml	(Han et al. 2018)
Gold Nanorods	Antibody	Milk	*E. coli O157:H7*	Qualitative: Naked Eye Quantitative: portable strip reader (absorption wavelength = 525 nm)	15 min	1×10^2 cfu/ml	(Tao et al. 2018)
AuNPs + MNPs	Antibody	Water, milk, beef	*E. coli 0157:H7*	Qualitative: Naked Eye	< 1 h	1 x 10^3 CFU/ml	(Qi et al. 2011)
Plasmonic enhanced lateral flow sensor utilizing liposome	Antibody	Cranberry juice	*E. coli O157:H7*	Quantitative: analysis of signal intensity using image J	10 min	600 cfu/ml	(Ren et al. 2019)
Streptavidin-QDs	Antibody	Minced beef, Water	*E. coli O157:H7*	Quantitative: The detective results can be read by portable LFS readers or smartphones.	10 min	178 cfu/g (beef) and 133 CFU/ml (water)	(Hassan et al. 2019)
Surface-enhanced Raman scattering nanotags	Antibody	Beef, chicken breast, milk	*E. coli O157:H7*	Quantitative: Raman spectrometer	15 min	5×10^4 cfu/ml or cfu/g	(Liu, Chen, et al. 2019)
MNPs	peptide	Lettuce, milk, meat,	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: image analysis software (ImageJ)	30 sec	30-300 CFU/ml	(Suaifan, Alhogail, and Zourob 2017)
AuNPs + HAuC14	DNA	Buffer	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: Strip Reader	NA	0.4 nM	(Rastogi et al. 2012)
AuNPs	DNA	Buffer	*E. coli*	Qualitative: Naked Eye	25 min	5 x 10^4 CFU/ml	(Pöhlmann, Dieser, and Sprinzl 2014)
AuNPs	Antibody	meat products vegetables	*E. coli O26*	Qualitative: Naked Eye	18 h	2.2 x 10^3 CFU/ml	(Yonekita et al. 2013)
Smartphone-based LFIA imaging system	Antibody	Ground beef, spinach	*E. coli O157:H7*	Quantitative: Smart Phones	15 min	10^4–10^5 cfu/g	(Jung et al. 2020)
Reduced graphene oxide	Antibody	Milk	*E. coli O157:H7*	Quantitative: strips were irradiated, and the temperatures before and after irradiation were recorded with an IR camera.	25 min	5×10^5 cfu/ml for standard bacteria solutions	(Shirshahi et al. 2020)
Gold superparticles added to polymer nanobead	Antibody	Milk	*E. coli O157:H7*	Quantitative: HG-8 strip reader	15 min	5.95×10^2 cfu/ml	(Li, Chen, et al. 2020)
Mannose Modiefied Perssiun Blue nanoenzyme	Antibody	Potable water, watermelon juice, milk and purple cabbage salad	*E. coli O157:H7*	Semi-quantitative	10 min	10^2 cfu/ml or cfu/g	(Wang, Yao, et al. 2020)
AuNPs	Antibody	Raw beef	*E. coli O157:H7*	Qualitative: Naked Eye	10 min	1.8×105 CFU/ml	(Jung, Jung, and Kweon 2005)

(*Continued*)

Table 3. Continued.

Probe	Receptor	Sample	Target foodbourne pathogen	Qualitative/Quantitative	Assay time	LOD	Refs
QDs	Antibody	Buffer milk	*E. coli*	Quantitative: Portable lateral flow reader	NA	1 x 10^1 CFU/ml (buffer) – 1 x 10^2 CFU/ml (milk)	(Morales-Narváez et al. 2015)
QDs	DNA Aptamer	Buffer	*E. coli*	Qualitative: UV lamp Quantitative: fluorescence instrumentation	15 min	300-600 cells	(Bruno 2014)
MNPs + fluorescent nanobeads (FNs)	Antibody	Milk	*E. coli 0157:H7*	Quantitative: fluorescent reader	5 h	1 x 10^2 CFU/ml	(Huang et al. 2016)
UCPs	Antibody	Buffer	*E. coli 0157:H7*	Quantitative: **UP*link*** reader	10 min	1 x 10^2 CFU/ml	(Niedbala et al. 2001a)
AuNPs	DNA	Food samples	*E. coli 0157:H7*	Qualitative: Naked Eye	18 h	1 x 10^6 CFU/ml	(Saad et al. 2015)
Carbon nanoparticles (CNPs)	DNA	Fruit juice buffer	*E. coli*	Qualitative: Naked Eye Qualitative: flatbed scanner and image analysis software	1 h	1-5 ng/ml	(Posthuma-Trumpie et al. 2012)
CNPs	DNA	Buffer	*E. coli*	Qualitative: Naked Eye Qualitative: flatbed scanning	70 min	6.7 x 10^4 CFU/ml	(Noguera et al. 2011b)
AuNPs + HAuC14	Antibody	Milk	*E. coli 0157:H7*	Qualitative: Naked Eye	20 min	5 x 10^3 CFU/ml	(Wang, Chen et al. 2015)
Dual AuNPs	Antibody	Buffer milk	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: CG-ICTS reader	15 min	1.14 x 10^3 CFU/ml – 1.14 x 10^4 CFU/ml	(Chen et al. 2015)
AuNPs	Antibody	Buffer	*E. coli 0157:H7S. aureus*	Qualitative: Naked Eye Quantitative: optical density analysis.	NA	1 x 10^6 CFU/ml	(Park, Shin, and Park, Ho Shin, and Park 2016)
AuNPs	Antibody	Buffer	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: optical density analysis.	15 min	1 x 10^3 CFU/ml	(Zhang et al. 2015)
AuNPs + HRP-TMB	Antibody	Beef	*E. coli 0157:H7*	Qualitative: Naked eye Semi-quantitative: signal intensities	15 min	1 x 10^2 CFU/ml	(Cho, Bhunia, and Irudayaraj 2015)
Pt-AuNPs + TMB	Antibody	Buffer	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: commercial assay meter	10-20 min	10^2 cells/ml	(Jiang et al. 2016)
AuNPs + MNPs	DNA Aptamer	Buffer	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: handheld strip reader	NA	1 x 10^1 CFU/ml	(Wu, Milutinovic, et al. 2015)
AuNPs	Antibody	Buffer	*E. coli 0157:H7*	Qualitative: Naked Eye Quantitative: FlexiBioAssay Reader	10 min	1 x 10^2 CFU/ml	(Cui et al. 2015)
AuNPs	Antibody	Buffer	*Pseudomonas aeruginosa; S. aureus;*	Qualitative and Semi-Quantitative: voltage reading using biosensor	10 min	500-5000 CFU/ml	(Li et al. 2011)
AuNPs	CBD	Buffer	*B. cereus*	Qualitative: Naked Eye Quantitative: ImageJ software	20 min	1 x 10^4 CFU/ml	(Kong et al. 2017)
UCPs	Antibody	Buffer	*Yersinia pestis; Burkholderia pseudomallei*	Qualitative: Naked Eye Quantitative: UPT biosensor designed and fabricated by the authors and Shanghai Institute of Optics and Fine Mechanics	NA	1 x 10^3 CFU/test	(Liang et al. 2017)
MNPs	Antibody	Dairy products	*B. anthracis*	Qualitative: Naked Eye	40 min	5 x IO^5 CFU/ml	(Fisher et al. 2009b)

nano-gold in the form of silver under the action of the reducing agent. Silver enhancement technology has been frequently used in immunogold assays to amplify the signal of the colloidal gold probe (Rodríguez et al. 2016). Table 3 summarize LFA studies in which AuNPs were used for the detection of foodborne pathogens.

Magnetic nanoparticles (MNPs)

Recently, iron oxide magnetic nanoparticles (MNPs) have received interest as promising materials for rapid and high-sensitive diagnosis methods due to their unique properties (Xu et al. 2019). When an external magnet is present, MNPs can function as "nano-magnets" in the system. They move very quickly in the direction of the external magnet, but as soon as the magnetic field is removed, they lose all of their magnetism (Ha et al. 2018). In addition, it is a well-established fact that MNPs are able to retain their magnetic properties while forming stable conjugates with a wide range of biomaterials. Due to these properties, MNPs enable magnetic pre-concentration of a target from a very diluted concentration *via* two steps: (1) targets interact with bioconjugated MNPs, and (2) target-bioconjugated MNPs are collected with an external magnet and re-dispersed into a small volume of matrix for the pre-concentration of samples to improve the detection sensitivity. Most importantly, MNPs offer low background noise since the biological materials that do not interact with MNPs are non-magnetic (Gowri, Ashwin Kumar, and Suresh Anand 2021). Furthermore, MNPs have a high surface area, which enables quick interaction between the target and antibodies conjugated to MNPs, which in turn reduces the amount of time required for detection. Therefore, MNPs have been successfully used for the quick and extremely sensitive detection of a wide variety of target analytes, ranging from viruses (Castilho et al. 2011; Sánchez-Cano et al. 2021) and bacteria (Pappert et al. 2010; Mun and Choi 2015) to food allergens (Speroni et al. 2010; Yin et al. 2022).

Because MNPs are capable of transmitting both an optical signal and a magnetic signal, they make excellent labels for LFAs. Their dark color allows them to be used as conventional optical labels, and their magnetic field enables easier functionalization, sample pretreatment (Nash et al. 2012), and sensitive readout. Although optical readouts rely heavily on labels on the NC, magnetic field sensing permits the use of all labels collected along the test line (Quesada-González and Merkoçi 2018). The major advantage of MNPs is that they can be detected and quantified by means of external devices, allowing the quantitative detection of the target. However, MNPs are more expensive to use compared to AuNPs. Table 3 summarize LFA studies in which MNPs was used.

Carbon nanoparticles

Since their discovery in 1991, Carbon nanoparticles (CNPs) (Mao et al. 2009; Noguera et al. 2011a) and carbon nanotubes (Qiu et al. 2015; Yao et al. 2016) have emerged as one of the most promising nanomaterials for the development of biosensors (Iijima 1991). In addition to their large surface area, carbon nanotubes exhibit superior electrical conductivity, mechanical strength, and chemical inertness (Ajayan 1999). Most Importantly, Carbon nanoparticles are preferred for their high signal-to-noise ratio (black to a white background) (Mens et al. 2008; Amerongen, Barug and Lauwaars 2005) and their excellent sensitivity, i.e., low picomolar by visual inspection (Gordon and Michel 2008). Further, they are inexpensive to produce, resistant to aggregation, and easy to functionalize. Carbon nanotubes are known to have a high surface specificity and allow various alterations with functional groups enabling sensitive protein recognition based on electron transfer processes. In addition, carbon nanotubes have been shown to have excellent chemical stability. This has led to the development of CNT-based sensor systems for biorecognition, diagnostics, and therapeutic purposes such as DNA sensors (Sánchez-Pomales, Santiago-Rodríguez, and Cabrera 2009), chemical sensors (Kong et al. 2000), and immunosensors (Okuno et al. 2007). Table 3 summarize LFA studies in which carbon nanoparticles were used.

Fluorescent nanoparticles

Fluorescent nanoparticles (FNs) are frequently recommended for the detection of targets at low concentrations and/or quantitative applications. This incurs extra costs and necessitates the use of an external reader. Quantum dots (QDs), upconverting nanoparticles (UCNPs), and liposomes encapsulating fluors are examples of fluorescent LFA reporters. QDs, also known as fluorescent semiconductor nanocrystals, can be used to develop highly sensitive LFAs because of their bright signal, resistance to photobleaching (Yan et al. 2016; Bruno 2017), chemical and thermal stability, and ease of surface modification (Wang, Meng, et al. 2019). The size of QDs ranges from 1 nm to 10 nm, which enables them to disperse well in water and to be combined with biomolecules. When activated by UV light, QDs display intense photoluminescence, which can be adjusted by modifying the elemental composition and size of the QDs. Therefore, QDs are appropriate for multiplexed detection (Medintz et al. 2005; Wang, Shen, et al. 2020). Nevertheless, the formation of QD-biomolecule complexes are difficult, resulting in rendering their application compared to AuNPs (Costa-Fernández, Pereiro, and Sanz-Medel 2006).

UCNPs have been employed as labels in LFAs since the early 2000s (Niedbala et al. 2001b) (Corstjens et al. 2001; Hampl et al. 2001). Their near-infrared excitation wavelengths do not generate membrane autofluorescence, and their strong emission in the visible spectrum makes them more sensitive than QDs as detection molecules in LFAs (Kim et al. 2018). However, the need for an expensive and bulky near-infrared laser makes the incorporation of UCNPs into LFAs impractical for many on-site applications (Kim et al. 2018; He et al. 2018; You et al. 2017) (Gong et al. 2019).

Many studies have examined the use of fluorescent dyes added to liposomes to increase the sensitivity of LFAs. Liposomes can be combined with a wide range of

fluorescent dyes capable of producing a strong fluorescence signal, and the composition of the lipid bilayer can be altered to permit straightforward bioreceptor functionalization of the liposome surface (Khreich et al. 2008; Baeumner et al. 2004; Edwards and Baeumner 2006; Edwards, Korff, and Baeumner 2017). The two fundamental disadvantages of liposomes as LFA indicators are their complex production processes and poor stability. Table 3 summarize LFA studies in which fluorescent nanoparticles were used.

Improving the sensitivity and specificity of LFAs

Selection of the best antibody pair

The specificity of LFAs can be increased by reducing non-specific binding (NSB) and by using antibodies with high affinity for the analyte. Several sample processing techniques are available to reduce NSB, which leads to false-positive findings. For example, when detecting analytes from whole blood, blood cells and large proteins are often removed from the blood by filtering or centrifugation prior to LFA (Liu et al. 2021). It was also shown that preheating urine decreased the activity of thermally unstable biomolecules, resulting in fewer false-positive findings when LFA was used to detect cryptococcal antigens (Nam, Thaxton, and Mirkin 2003).

The LFA efficacy is heavily dependent on the affinity proteins (i.e., antibodies) that recognize the target. Maximum specificity can be achieved by molecules with optimal affinity (Bembenek et al. 2011; Brooks et al. 2008; Wu, Milutinovic, et al. 2015). Antibodies are a typical option because of their sensitivity as well as their specificity when it comes to the specific detection of very low concentrations of the analyte. While aptamers and various other affinity reagents are also options, antibodies are the primary affinity reagent used for lateral flow rapid tests.

The selection of the optimal antibodies is a critical aspect of LFA design. The ultimate performance of the LFA mainly depends on the specificities of the antibodies used to bind a target in the specimen. The decision of whether to utilize polyclonal or monoclonal antibodies is one of the earliest decisions that must be made in the process of LFA development. Polyclonal antibodies are derived from the serum of animals that have been vaccinated. They are made up of complex mixtures of antibodies, each of which was created by a unique B cell clone in the animal. There is an inherent lack of consistency from one animal to the next, and even fluctuation from one bleed of the same animal to the next since every host species and even every individual host will have a distinct immunological response. On the other hand, monoclonal antibodies are produced in the laboratory; thus, they are homogenous. Monoclonal antibodies are unique in that they are only able to bind to a single epitope of the target and were generated by a single B cell clone. Therefore, polyclonals may have a stronger recognition ability owing to multiple kinds of antibodies targeting different epitopes of the target, but monoclonals are more consistent since they target just one epitope of the target. This is because polyclonals target numerous antigens simultaneously. An additional benefit is that the cell clones that are used in the production of monoclonal antibodies may be regenerated endlessly in the laboratory, but the animal hosts that are utilized in the production of polyclonal antibodies will ultimately perish.

Extensive efforts have been made to enhance the sensitivity and specificity of LFAs for more precise and effective on-site diagnostics. Assay optimization and sample enrichment are two ways to increase sensitivity (Soh, Chan, and Ying 2020; Nguyen et al. 2020; Bishop et al. 2019). Signal amplification can boost LFA sensitivity close to that of PCR-based assays. Several other approaches to improve LFA sensitivity are promising but require extended testing time (Bishop et al. 2019; Rodríguez et al. 2016). Hence, balancing sensitivity and test duration is a critical challenge for the future development of on-site assays. LFA specificity is primarily increased by optimizing the test and applying high-affinity antibodies and reagents with high specificity.

Improving sensitivity by assay optimization

Signal amplification

Chemical enhancement of colorimetric signal. The LFA sensitivity can be improved by increasing the colorimetric signal of the test. A quick and easy way to boost the signal is by chemically increasing the colorimetric contrast of the positive test line. This enhanced contrast can be achieved using different methods, including; silver enhancement, double gold conjugation, and induced gold aggregation (Liu et al. 2021). In the silver enhancement method, Ag-reducing reagents are flowed through the LFA strip after running the sample, and Ag is nucleated on captured AuNPs in the test area. The Ag layer forming on the AuNPs reporter particles amplifies the color intensity of the test area. This method significantly enhances the sensitivity by 10-fold compared to traditional LFA (Serebrennikova, Samsonova, and Osipov 2018; Anfossi et al. 2013). For the double gold conjugation, secondary AuNPs are used to bind with the primary AuNPs that are already captured on the test area leading to enhanced color intensity. This binding can be accomplished through the utilization of the high biotin-streptavidin binding affinity (Shen and Shen 2019) or by employing primary and secondary antibodies, which is similar to the basis of indirect ELISA. It was shown that the double gold conjugation method has significantly increased the sensitivity of LFA by approximately 30-fold for the detection of the Hepatitis B virus (Shen and Shen 2019). The concept of the induced gold aggregation technique is similar to the double gold conjugation approach. However, more AuNPs are coated on the captured AuNPs, thus better amplifying the color intensity.

Label design. The label design plays an essential role in amplifying the colorimetric contrast of LFA. Replacing the traditional small (20 – 40 nm) nanoparticles employed with labels that have stronger colorimetric contrast is the easiest way to enhance the signal while maintaining the LFA format. Stronger contrast can be achieved by modifying the size and structure of the reporter particles or by replacing the particles with clusters or particles made of another metal, metal oxide, or organic material. For instance, gold-nanoparticle-decorated silica nanorods (AuNPs -SiNRs), achieved a 50-fold lower LoD in the detection of rabbit IgG than traditional LFA with AuNPs (Xu et al. 2014). Similarly, polystyrene microbeads were used to enhance the colorimetric contrast of AuNPs. Utilizing polystyrene microbeads improved the sensitivity of LFA for the detection of influenza virus by 64-fold and 16-fold over that achieved with 10 nm and 30 nm AuNPs -based LFAs, respectively (Liu et al. 2020).

Enhancing LFA reagents. Enhancing LFA reagents can be used to induce catalytic reactions in the test area to amplify the signal contrast. Catalytic amplification is usually achieved by utilizing enzymes or nanozymes to induce oxidation/reduction reactions in the test area. HRP is one of the most commonly used enzymes in LFA platforms. In LFA, HRP is linked to detection molecules (i.e., antibodies) that are conjugated with reporter particles immobilized on the conjugation pad. After the sample flow through the LFA is completed, the HRP substrate and H2O2 solution are flowed through the LFA after washing to induce enzymatic amplification and enhance the optical contrast at the test line (He et al. 2011). Parolo et al. reported an increase in sensitivity up to 1 order of magnitude compared to traditional AuNPs -LFAs by applying enzymatic amplification (Parolo, de la Escosura-Muñiz, and Merkoçi 2013).

Nanozymes, which are artificial enzymes based on nanomaterials, were rapidly developed as surrogates of natural enzymes. Nanozymes have several advantages over natural enzymes, including higher catalytic stability, an easier modification process, and lower manufacturing costs (Jiang et al. 2019). For instance, thin platinum (Pt) shells on top of gold (Au) nanoparticles (NPs) (Au@PtNPs) was able to produce an LoD of 0.8 pg/mL. This finding is significantly more sensitive than commercial ELISA, which has an LoD of >1 pg/mL (Loynachan et al. 2018).

External readers. Amplification of LFA signals may also be achieved with the assistance of external readers. When labeled particles that change in color intensity are used in LFA, a charge-coupled device or a complementary metal-oxide-semiconductor camera detection device will be utilized for assay quantification (Gussenhoven et al. 1997). In case of using fluorescent labeled particles, a photodetector with an excitation light source is used for assay quantification (Ho and Wauchope 2002). The laser beam, or electric potential, or magnetic field can be used to activate/concentrate captured nanoparticle labels on the test line, resulting in an enhanced signal (Draz and Shafiee 2018). This amplified signal can then be detected by sensitive optical, electrical, or magnetic sensors/electrodes, respectively, that can distinguish between minute signal variations and background noise. Among these, LFA readers utilizing image sensors, such as a charge-coupled device or metal-oxide-semiconductor camera, are most commonly used because of the advantages of their simple structure and small size (You, Park, and Yoon 2013). An image sensor-based LFA reader acquires an image of the test line (aggregated labeled particles, antigens, and antibodies). Then, the pixel intensity of the test line, which changes according to the concentration of the target analyte, is analyzed (Sajid, Kawde, and Daud 2015). However, problems such as the high possibility of false positives and false negatives and limitations for accurate and multiplex quantification have been observed in the utilization of optical readers.

Recently, our team have designed and developed an ultra high-sensitivity inductive transducer, called the Femtogmag, for the detection and quantification of superparamagnetic nanoparticle reporters that are immuno-captured on the test line (Khodadadi et al. 2019). As a proof of concept, the femtoMag was used to quantify the hCG pregnancy hormone by quantifying the number of 200 nm magnetic reporters immuno-captured within the test line of the LFA strip. A sensitivity of 100 pg/mL has been demonstrated. Upon further design and control electronics improvements, the sensitivity is projected to be better than 10 pg/mL. Magnetic reporters provide several advantages compared to other optical reporters (1) Magnetic fields do not interact with biological materials, so the signal is stable (2) magnetic fields are not affected by LFA media, so every magnetic reporter within the test line contributes to detection; and (3) the properties of magnetic reporters can be tuned to match the biomarkers to optimize trapping efficiency and detection (Yoshino, Maeda, and Matsunag 2010; Yu et al. 2022; Jacinto et al. 2018). The femtoMag also provides a number of technological advancements over the current state-of-the-art magnetic biosensor technologies, including (1) high sensitivity, (2) Quantitation, (3) simple and easy integration with LFA technology, (4) portable electronic controls, and (5) low-cost manufacturability. The low-cost easy-to-use femtoMag platform offers high-sensitivity/high-precision target analyte quantification and promises to bring state-of-the-art medical diagnostic tests to the POC.

Optimization of the assay kinetics

Optimization of assay kinetics, such as transport and reaction kinetics, is essential for LFA development and can be used to increase sensitivity. The assay kinetics affect the selective binding (SB) and NSB of the antibodies and analytes, which determine the sensitivity and specificity of the

assay (55-57). Assay kinetics should be optimized to increase SB and reduce NSB, thus, enhancing the assay sensitivity (Zhan et al. 2020).

The sensitivity of LFAs is limited by the reaction rate (Zhan et al. 2017b; Mosley et al. 2016). Increasing the reaction rate can help in boosting assay sensitivity. Hence, maximizing the SB. However, the transport of molecules and labels is limited by the diffusion rate, and the surface reaction is limited by the reaction rate. Increasing the reaction kinetics is associated with the formation of the sandwich ternary; conjugation/target/capture antibody. Liang et al. showed that the sandwich ternary develops more slowly when the target binds first with the conjugated label and then the capture antibody in a premixing flow compared to binding with the capture antibody and then the conjugated label in a sequential flow. For instance, the LoD of the LFA platform for detecting malarial antigens from a premixing flow was reported to be 4- to 10-fold higher than that obtained from a sequential flow (Liang et al. 2016).

Improving sensitivity by sample enrichment

The reaction rate coefficient is relatively constant for most immunoreactions between antigens and antibodies. It is possible to effectively increase the reaction rate and, as a result, boost the sensitivity by increasing the number of captured labels on the test area by preconcentrating the food sample before introducing the sample into the LFA test. Magnetic separation is one technique that may be used for pre-concentration, which produces a 10-fold increase in sensitivity (Sharma et al. 2019). Alternatively, Mashayekhi et al. used an aqueous two-phase (Bradbury et al. 2019) micellar system composed of the nonionic surfactant Triton X-114 to concentrate a model protein and reported a 10-fold increase in sensitivity from 0.5 µg/mL to 0.05 µg/mL (Mashayekhi et al. 2012).

Analytes can also be preconcentrated during the LFA flow phase. The comparatively low LoD of LFAs may be attributed to the fact that low target concentrations induce kinetically limited surface reactions. To overcome this problem, Moghadam et al. preconcentrated the antigen-conjugation complex into a narrow band and transported it to the capture line using the isotachophoresis technique. This approach increased the LoD by 400-fold and 160-fold for 90 s and 5 min reaction, respectively (Moghadam, Connelly, and Posner 2015).

Another way to boost the reaction rate and enhance the sensitivity is to increase the number of efficient binding sites for the conjugated labels by altering the structure of the label or changing the orientation of the detection molecules. For instance, the number of binding sites can be increased by increasing the size of AuNP labels (Zhan et al. 2017a) or by functionalizing the particle surface with several layers (Lou et al. 2019) to allow for the loading of additional detection molecules.

Improved conjugation approaches have the potential to offer more effective binding sites than standard physical adsorption when the orientation of the detection molecules is forced in a specific direction (Trilling, Beekwilder, and Zuilhof 2013; Di Nardo et al. 2019; Welch et al. 2017). A particular orientation can be achieved through covalent binding mediated by a chemical layer or through bioaffinity binding mediated by a biomolecular layer (Trilling, Beekwilder, and Zuilhof 2013; Di Nardo et al. 2019; Welch et al. 2017). It is also necessary to adjust the coverage of the detection molecules in order to maximize the affinity for the analyte and get rid of any steric hindrance that may be generated by a thick layer of detection molecules (Saha, Evers, and Prins 2014). Another way to boost the sensitivity of the LFA is to add more effective binding sites to the test line. For instance, the use of three-dimensional "proteinticle" probes with multiple self-assembled and orientated peptides was shown to give a 4-fold to 8-fold improvement in sensitivity (Lee et al. 2015). In a different approach, the addition of cellulose nanofibers to the NC enabled more capture molecules to be loaded and increased the assay sensitivity by 20-fold (Tang et al. 2019; Quesada-González et al. 2019).

Commercially available LFAs for the detection of foodborne pathogen

The benefits of LFAs for the detection of food pathogens include multiplexing capabilities, dependability, and adherence to the same standards of precision as traditional detection techniques. In addition, LFAs are user-friendly and capable of producing qualitative, semiquantitative, or quantitative results after only a few (10–30) minutes. Most importantly, LFAs are economical, as their rapid findings save operational costs by accelerating product release while maintaining product dependability. Table 4 summarizes the characteristics of commercially available LFAs for the detection of foodborne pathogens.

Recent advances in LFA-based testing for foodborne pathogens

Bacterial foodborne pathogens

Bacteria are the most abundant type of foodborne pathogens that threaten human health (Mead et al. 1999; Hariram and Labbé 2016). Hence, the detection of foodborne bacterial pathogens is of supreme importance to guarantee food quality. Biochemical characterization and microbiological identification are used as conventional methods to detect foodborne bacteria (Byrne et al. 2015). Recently, LFA was shown to be a quick and sensitive alternative approach to identifying foodborne pathogens (Keiser and Utzinger 2005; Law et al. 2014; Hwang et al. 2016; Zhao et al. 2016a; Li et al. 2021). Table 3 lists recent studies on LFAs for the detection of foodborne bacterial pathogens.

Anthrax is an infectious disease caused by *Bacillus anthracis (B. anthracis)*. A combination of immunomagnetic separation and LFA has been used to detect *B. anthracis* in milk (Fisher et al. 2009a; Wang, Tian, et al. 2015). Fisher et al. (Fisher et al. 2009a) studied the immunocapture of *B. anthracis* spores using anti-spore antibodies coupled with

Table 4. Summary of commercially available LFA for detection of foodborne pathogens from food samples.

Target	Test	Sample Type	LoD/TN/TP	Type	Quality	Reporter	Results	Storage temp	Approval	Ref
Bacillus cereus enterotoxin	Duopath® Cereus Enterotoxins; Merck Millipore (104146)	Food and beverages, including; meat and milk products, vegetables, soups, spices and baby food.	1 CFU/NR/NR	Q	NR	Gold labeled antibodies	30 min	2–8 °C	NR	(Millipore 2023a)
Campylobacter	Singlepath® Campylobacter Rapid test; Merck Millipore (104143)	Food and beverages	10^4–10^7 CFU/98%/100%	Q	MQ200	Gold labeled antibodies	20 min	2–8 °C	AOAC	(Millipore 2023b)
Salmonella	Singlepath® Salmonella; Merck Millipore (104140)	Food and beverages	1 CFU/25 g food/NR/NR	Q	MQ200	Gold labeled antibodies	20 min	2–8 °C	AOAC	(Millipore 2023e)
E. coli O157:H7	Singlepath®*E. coli* O157; Merck Millipore (104141)	Food and beverages	1 CFU/>99%/>99%	Q	MQ200	Gold labelledd antibodies	20 min	2–8 °C	AOAC	(Millipore 2023c)
Listeria monocytogenes/*Listeria* genus	Singlepath® L'mono; Merck Millipore (1041480001)	Food and beverages	5×10^6 CFU/NR/NR	Q	MQ200	Gold labelleded antibodies	30 min	2–8 °C	AOAC	(Millipore 2023d)
Listeria	VIP® Gold Listeria; BioControl (60037-40)	Food and beverages	NR/NR/NR	Q	NR	Gold labelleded antibodies	10 min	RT	AOAC	(Millipore 2023g)
Salmonella	VIP® Gold for Salmonella; BioControl (60038-40)	Food and beverages	NR/NR/NR	Q	NR	Gold labelledled antibodies	10-15 min	RT	AOAC	(Millipore 2023h)
E. coli O157:H7	VIP® Gold EHEC; BioControl (60036-40)	Food and beverages	NR/NR/NR	Q	NR	Gold labelledled antibodies	a. 15–20 min for the 10 h raw meat protocol b. 10 min for the 18 h pasteurized juices protocol	RT	AOAC	(Millipore 2023f)
E. coli O157:H7	RapidChek® E. coli O157 test strip; Romer Labs (Austria);10001355	Meat, dairy, and plant products.	6cfu/6 g of sample	Q	NR	Gold Particles	10 min	15 °C – 25 °C	AOAC	(Labs 2023)
E. coli O157:H7	MaxSignal- E. coli O157 Test Strip Kit; BIOO Scientific Corp. (Japan); BO 1062-01 / 02	Variety of food matrices, including meat and dairy products	1×104 CFU - 1×105 CFU.	Q	NR	NR	10 min	4 °C	Not certified	(Corp 2023)
E. coli O157:H7	DuPont™M Lateral Flow System E. coli 0157 Test Kit; Hygiena, LLC (CA, USA); ASY2030;	Beef and acid cidar	NR/100%/100%	Q	NR	Gold nanoparticles	10 min	2-8 °C	AOAC	(Hygiena 2023)
Aflatoxin	AuroFlow™ AQ Afla Strip Test; Perkin Elmer (FOOD-1413-01)	Corn	2–300 ppb/NR/NR	N	NR	NR	4–6 min	NR	USDA certifications	(Elmer 2023f)
DON	AuroFlow AQ DON Strip Test; Perkin Elmer (FOOD-1414-01)	Corn and Wheat	0–30 ppm/NR/NR	N	NR	NR	4–6 min	NR	USDA certifications	(Elmer 2023a)
Zearalenone	AuroFlow AQ Zearalenone Strip Test; Perkin Elmer (FOOD-1415-01)	Corn and Wheat	NR/NR/NR	N	NR	NR	4–6 min	NR	USDA certifications	(Elmer 2023e)
Fumonisin	AuroFlow AQ Fumonisin Strip Test; Perkin Elmer (FOOD-1416-01)	Corn	0–30 ppm/NR/NR	N	NR	NR	4–6 min	NR	USDA certifications	(Elmer 2023b)
Ochratoxin A	AuroFlow AQ Ochratoxin A Strip Test; Perkin Elmer (FOOD-1417-01)	Wheat	2–100 ppb/NR/NR	N	NR	NR	4–6 min	NR	Not Certified	(Elmer 2023c)
T-2/HT-2	AuroFlow AQ T-2/HT-2 Strip Test; Perkin Elmer (FOOD-1418-01)	Corn and Wheat	50–500 ppb/NR/NR	N	NR	NR	4–6 min	NR	Not Certified	(Elmer 2023d)

NR: Not Reported; TP: True Positive (Sensitivity); TN: True Negative (Specificity); Q: Qualitative; N: Quantitative; RT: Room Temperature; AOAC: Association of Official Agricultural Chemists.

carboxylated magnetic beads and were able to recover 95% of *B. anthracis* spores when 10^5–10^7 spores were inoculated in 1 mL milk (Fisher et al. 2009a).

E.coli is the etiological agent of many waterborne and foodborne diseases (Singh, Sharma, and Nara 2015a). Wu et al. used an aptamer-based biosensor to rapidly detect *E. coli* O:157:H7 (Wu, Milutinovic, et al. 2015). This assay utilized two distinct aptamers, each designed to precisely target the outer membrane proteins of the bacterium. The first aptamer enriched *E. coli* O:157:H7 cells on magnetic beads, and the other was used as a signal reporter. The signal produced by the second aptamer was amplified using an isothermal strand displacement amplification technique. Positive signals, generated as red bands on the test line, could be produced by 10 CFU/mL of *E. coli* O157:H7 (Wu, Milutinovic, et al. 2015). Bruno et al. (Bruno 2014) used a sandwich-format LFA sensor to detect *E. coli*. In this system, amino end-labeled capture aptamers were immobilized on an analytical NC using UV light with a wavelength of 254 nm. When *E. coli* cells passed through the capture line, they were captured by the amino group on the membrane surface. The aptamer-conjugated colloidal gold demonstrated a visible LoD between $3\times10^3$and 6×10^3 *E. coli* cells in the buffer (Bruno 2014).

Liu et al. developed a simple and ultra-sensitive LFA for the rapid recognition of *Salmonella* in food samples. They used AuNPs conjugated with a DNA probe that was complementary to the 16S ribosomal DNA and RNA of *Salmonella*. The synthesized single-stranded DNA had an LoD of five femtomolar. For cultured *Salmonella*, the nucleic acids of 10^7 bacteria were rapidly detected in 30 min. Additionally, with silver enhancement, the LoD was improved to detect 10^4 bacteria, which is lower than the human infectious dose of foodborne *Salmonella* (10^5 CFU) mL-1. Because of its low cost, high sensitivity, high specificity, and ease of use, the LFA developed by Liu et al. may be a valuable tool for microbial detection in large-scale diagnostic or food safety applications in impoverished nations (Liu et al. 2013b).

Wang et al. developed an LFA strip biosensor that could detect *Salmonella enteritidis* (S. *enteritidis*) with an LoD of 10^2 CFU/mL using positively charged, surface nitrogen-rich, carbon nanoparticles (pNPs) made by calcination and etching procedures (Wang, Yao, et al. 2019). These nanoparticles not only generate a signal but also function as an adsorbent to trap bacteria (Wang, Yao, et al. 2019). Bacterial cells stick to the pNCs by electrostatic contact and hydrogen bonding, and this complex is then selectively recognized by an anti-bacterial antibody coated on the test line, causing the color of the test line to progressively darken. The pNPs were able to recover 85–100% of *Salmonella* from various food specimens; however, the generalizability of the approach is currently limited by the availability of suitable antibodies (Wang, Yao, et al. 2019). Taking advantage of simplicity, label-free, convenience, and sensitivity, the pNC-based LFA has the application potential for pathogenic microorganisms monitoring in food safety and early clinical diagnosis fields (Wang, Yao, et al. 2019).

Viral foodborne pathogens

The LFA research and commercialization for the detection of viral foodborne pathogens are more hindered compared to those for bacterial foodborne pathogens. The bacterial detection techniques cannot be used for viral detection due to many factors; (1) viruses are far more complicated to culture and amplify compared to bacteria, which often cannot be enriched (Chhabra and Vinjé 2016), (2) viruses are usually present in small quantities in food matrices and cannot proliferate in host-free environment, (3) the size of viruses range from 20 to 400 nm, while the size of bacteria ranges from 1 micron to 5 microns. Therefore, the sensing platforms developed for bacterial detection must be significantly adjusted in order to identify viral pathogens due to their extremely smaller size. (4) the composition of cell surface proteins of bacteria is not comparable with the hemagglutinin and neuraminidase compositions of viruses, and this necessitates the selection of specific biorecognition elements for the detection of viral-specific proteins, and (5) viruses are often present with lower copy numbers compared to bacteria in food samples matrix, which demands superior sensitivity of the virus detection biosensors to be at least attomolar or picomolar level (Neethirajan et al. 2017). Therefore, separation, pre-concentration, and purification of viral pathogens from food samples are crucial toward sensitive virus detection using LFA. Nevertheless, due to the heterogeneity in genome and surface structures among viruses, a universal viral extraction technique would be extremely difficult for the on-spot rapid and easy foodborne viral detection from food samples.

Our team is currently working to develop LFA for the detection of viral foodborne pathogens; Norovirus (NoV) and Hepatitis E (HEV). Using europium nanoparticles, we were able to detect NoV at a concentration as low as 10 ng/mL (Work in Progress). In addition, using AuNPs, we were able to detect HEV at a concentration as low as 5 ng/mL (Work in Progress). Currently, we are working to generate a novel quantitative magnetic immunoassay for the detection of foodborne pathogens, NoV and HEV. Using the femtoMag as a reader (Khodadadi et al. 2019), the LFA will have sensitivity rivaling those of central laboratory instruments, which will enable rapid, high-quality quantitation of viral levels and can serve as a simple, low-cost, easy-to-use point-of-need analytical platform for rapid and reliable early infection detection of foodborne pathogens associated with acute gastroenteritis from food samples.

Conclusion and future perspectives

Various contaminants threaten food quality and pose threats to human health. Most current methods to detect food contaminants are difficult to use on-site because they require special laboratory equipment and skilled personnel. LFAs offer many advantages for the rapid detection of foodborne pathogens, including cost-effectiveness, simplicity, rapidity, and ease of use in on-site settings. Another important advantage of LFAs is that they can analyze different analytes

simultaneously, which is of supreme importance. Current challenges for LFA platform development for the detection of foodborne pathogens include (1) enhancing the signal-to-noise ratio to reduce background noises and increase detectable signals (2) enhancing detection specificity and sensitivity; (3) improving storage duration; (4) allowing user-friendly and unskilled operation (Mangal et al. 2016).

With the changing of the global demographic and epidemiologic structure, as well as food processing and harvesting systems, We can expect new foodborne viruses to emerge in society through both animal and plant-derived foods. A rapid and sensitive detection system can reduce ongoing transmission of pathogens as well as play a crucial role in preventing pathogen transmission through early detection and control of foodborne illness outbreaks.

Various LFAs for food safety monitoring are commercially available; however, their widespread acceptance is hindered by their lack of sensitivity. The sensitivity, reproducibility, and multi-analyte capabilities of LFAs must be improved substantially for LFA-based food safety evaluation to be adequate.

Disclosure statement

Prof. Dmitri Litvinov is an executive officer of FemtoMag, Inc. FemtoMag is a for-profit company developing biosensor technologies for the detection and quantification of molecular biomarkers

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