



# Recent advances in sensitivity enhancement for lateral flow assay

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Received: 22 June 2021 / Accepted: 25 September 2021 / Published online: 13 October 2021  
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## Abstract

Conventional lateral flow assay (LFA) is typically performed by observing the color changes in the test lines by naked eyes, which achieves considerable commercial success and has a significant impact on the fields of food safety, environment monitoring, disease diagnosis, and other applications. However, this qualitative detection method is not very suitable for low levels of disease biomarkers' detection. Although many nanomaterials are used as new labels for LFA, additional readers limit their application to some extent. Fortunately, a lot of work has been done for improving the sensitivity of LFA. In this review, currently reported LFA sensitivity enhancement methods with an objective evaluation are summarized, such as sample pretreatment, the change of flow rate, and label evolution, and future development direction and challenges of LFAs are discussed.

**Keywords** Lateral flow assay · Sensitivity enhancement · Signal amplification

## Introduction

The rapid, portable, sensitive, and inexpensive detection of analytes from complex samples is essential for in vitro diagnostics [1, 2]. It is estimated that improving the technique of diagnostic tests for infectious diseases in developing countries can annually save at least 1.2 million deaths [3]. Especially when facing the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a rapid and facile screening strategy, which is employed in airports, customs, and community, shows the great significance to prevent epidemic and resume shipping and economic development.

Lateral flow assay (LFA) deployed as point-of-care testing (POCT), owing to its rapidity, simplicity, stability, and visual characteristics [4], has been vital in enabling faster diagnosis, directing medical interventions, and mitigating the transmission of infectious diseases [2].

Conventionally, the results of LFA are read out by naked eyes, by measuring the color change due to the accumulation of gold nanoparticles (AuNPs). This detection strategy is simple and rapid, and the early pregnancy test is the

outstanding representative. However, these results are qualitative and lack of sensitivity and may function only for certain applications. As for the detection of critical biochemical markers present in extremely small amounts in a sample, such as myocardial infarction and cancer, these methods do not afford sufficient sensitivity, which restrict their applications. In recent years, the development of new nanomaterials has broadened the type of labels available for LFA to enhance sensitivity. These nanomaterials can be roughly divided into three categories according to the type of readout [5], that is, naked-eye detection, fluorescence detection, and non-optical readout detection. Carbon nanoparticles [6, 7], carbon nanotubes [8, 9], and dye-loaded latex beads [10, 11] can provide an alternative to AuNPs for naked-eye detection. Fluorescent labels are generally recommended for low concentrations of targets and quantitative detection. Suitable fluorescent nanoparticles include fluorescent microspheres (FMs) [12], quantum dots (QDs) [11], upconverting nanoparticles (UCNPs) [13], and liposomes with fluorescent dyes [14]. LFA with non-optical readout can be comparable with that of LFA with fluorescent labels, such as magnetic nanoparticles [15–17] and nanoparticles for electrochemical readings [18–20]. However, they either cannot provide a strong signal as AuNPs for naked-eye detection or comes with a higher cost and the need for an external reader.

In order to enhance the signal–noise ratio, a lot of signal amplification methods have been employed in POCT, such

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as gas-propelled [21, 22], enzyme-mimicking accelerated signal enhancement [23, 24], and cascade amplification [25, 26]. As for AuNP-based LFA, significant efforts have been focused on highly sensitive detection, such as sample pretreatment, changes in structure, materials, and labels. In this review, currently reported LFA sensitivity enhancement methods with an objective evaluation are summarized, and future development direction and challenges of LFAs are also discussed.

### Sensitivity enhancement based on sample pretreatment

Serum and saliva are the common sample matrix for disease diagnosis. AuNPs may aggregate together caused by the mixture of proteins, nucleic acids, and other substances in the matrix of serum and saliva [27], which interferes with the sensitivity and specificity for detection. To get rid of interfering components, a lot of sample pretreatment methods are introduced in LFA, such as isothermal amplification for nucleic acid detection [28] or sample enrichment for proteins detection [29]. The following sections will highlight such sample pretreatment integrated with LFA.

### Sensitivity enhancement based on isothermal nucleic acid amplification

Comparing with other biomarkers, nucleic acids are more stable under harsh environments and are gradually classified as biomarkers for disease diagnosis [30], microbial detection [31], and environmental monitoring [32]. With an increasing demand for diagnosis under resource-limited conditions, LFA for nucleic acid detection has gained greater attention owing to the lower cost and user-friendly [33]. However, one major drawback of the current LFA for nucleic acid detection is a low sensitivity, limiting its practical applications. Different from protein biomarkers present at the level of nanomolar or picomolar, the amounts of pathogenic bacteria that cause disease can be as low as a few CFU/mL [34]. In order to enhance the sensitivity of LFA for nucleic acid detection, various isothermal amplification methods are used, including rolling circle amplification (RCA) [35], loop-mediated isothermal amplification (LAMP) [36], recombinase polymerase amplification (RPA) [37], nucleic acid sequence-based amplification (NASBA) [38], helicase-dependent isothermal DNA amplification (HDA) [39], and hybridization chain reaction (HCR) [40].

### Sensitivity enhancement based on RCA

RCA is an isothermal enzymatic process where a short DNA or RNA primer is amplified to form a long single-stranded

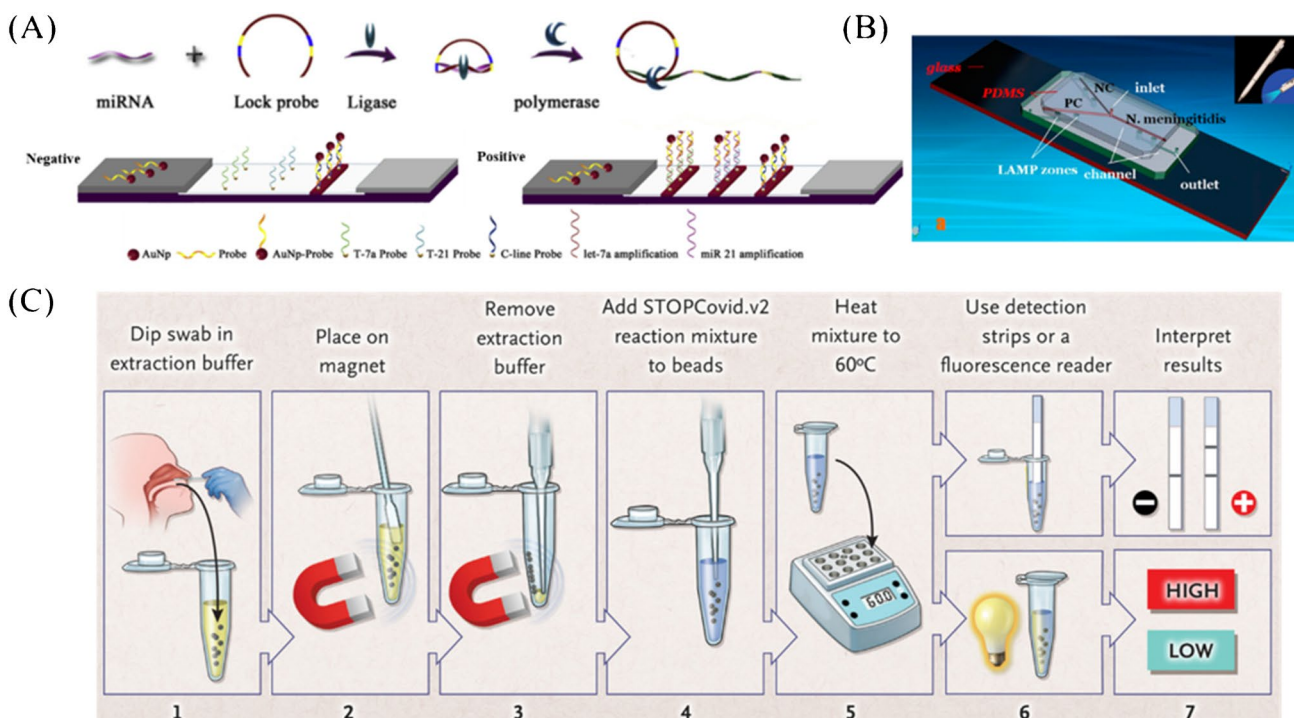
DNA or RNA under the function of a circular DNA template and special DNA or RNA polymerases [41, 42]. The RCA product is a concatemer containing tens to hundreds of tandem repeats that are complementary to the circular template [43], which gives a huge amount of capturing sites or signal generating sites. Yao et al. [35] combined RCA with AuNP-based LFA for simultaneous detection of miRNA 21 and miRNA let-7a. The limit of detection (LOD) was as low as 40 pM and 20 pM, respectively (Fig. 1A). Compared with the results obtained by Kor et al. [44], the sensitivity of miRNA21 was enhanced 7.5 times. Moreover, RCA integrated with LFA were designed for the detection of *Karenia mikimotoi* (hyperbranched RCA, HRCA [45]) and *Karlodinium veneficum* (exponential RCA, E-RCA [46]), respectively. Inspiringly, the sensitivity of HRCA-LFA was 100 times that of polymerase chain reaction (PCR) [45], and E-RCA-LFA was more sensitive than that of the conventional PCR and reached a LOD of 0.01 cell/mL [46]. In addition, RCA-based LFA has also been used in the detection of metal ions [47], small molecules [48], enzymes [49], and antibodies [50].

### Sensitivity enhancement based on RPA

RPA is regarded as an isothermal PCR method. Recombinase and single-stranded binding protein are used to replace the programmed temperature changing of PCR [53]. The recombinase is used to complete the dissociation of the template and the combination of primers and templates, while single-stranded binding protein is used for maintaining the single chain so that the DNA polymerase completes the primer extension process. Most importantly, it is an effective approach in terms of sensitivity, specificity, and multiplexing [54, 55].

Zhang's group [56] established a clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostic platform, which combined RPA and LFA with CRISPR-Cas enzymology for specific recognition of desired DNA or RNA sequences, called specific high-sensitivity enzymatic reporter unlocking (SHERLOCK). The template underwent the isothermal amplification reaction of RPA to amplify the signal. At the same time, a T7 promoter was added for in vitro transcription by RPA, which was used for converting the target from DNA to RNA for detection. After recognizing the target site of RNA, Cas13a would non-specifically cleave molecular beacons to generate signals. For RNA target, reverse transcription was necessary.

To date, RPA integrated with LFA has been widely used in the detection of pathogenic bacteria and viruses in food safety [57–59], environmental monitoring [37], and other biomedical fields [60, 61].



**Fig. 1** Sensitivity enhancement based on isothermal nucleic acid amplification. **A** Schematic illustration of RCA integrated with LFA for miRNA detection [35]. **B** Layout of the PDMS/paper hybrid

microfluidic device integrated with on-chip LAMP system [51]. **C** The whole process for STOPCovid, Version 2 (STOPCovid.v2) Test [52]

### Sensitivity enhancement based on LAMP

The core principle of LAMP is to design 2 or 3 pairs of primers for 6 regions of the target gene fragment to achieve rapid and efficient amplification [62]. Similar to nested PCR, multiple primers of LAMP strictly and efficiently ensured specificity. And, it is not affected by non-target DNA and PCR inhibitors with high similarity to the target DNA [63, 64].

Dou and co-workers [51] integrated polydimethylsiloxane (PDMS)/paper hybrid microfluidic chip with LAMP for *Neisseria meningitidis* detection (Fig. 1B). Compared with the results of previous studies [65], the introduction of paper into the microfluidic device for LAMP enabled more stable results than that of a paper-free microfluidic system. The LOD was about 3 copies of DNA, which was lower than that of other studies [66, 67].

Facing the COVID-19, Zhang’s group [52] developed an upgraded version of the new coronavirus detection process based on SHERLOCK, called STOPCovid (Fig. 1C). The RNA in the sample was enriched by adding magnetic beads during the sample preparation process, thereby increasing the amount of initial RNA in LAMP and further improving the detection sensitivity. In addition,

STOPCovid version 2.0 streamlined the operating steps of RNA enrichment with magnetic beads, removed the ethanol washing and eluting process, and shortened the whole time of the entire RNA enrichment process to less than 15 min.

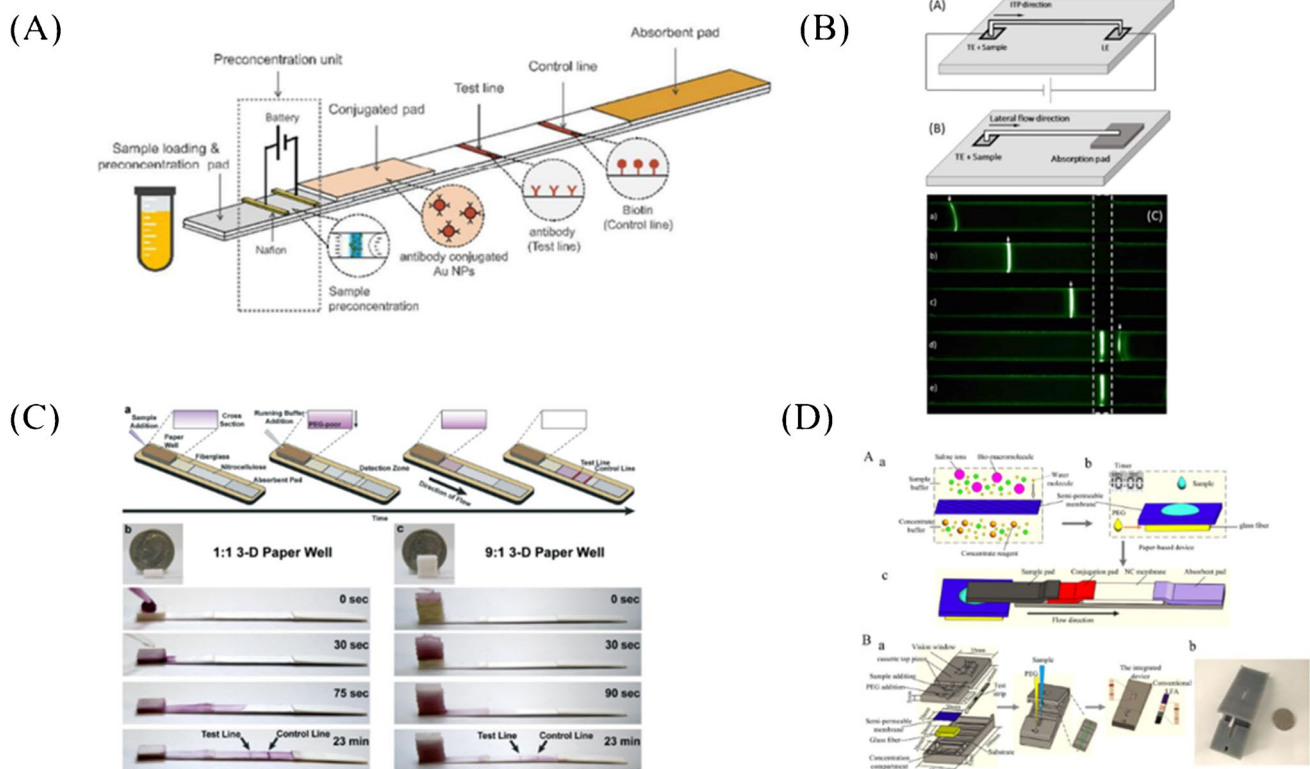
Furthermore, LAMP integrated with LFA has also been used in the detection of *E. coli* [68], plasmodium [64], *P. aeruginosa* [69], and SARS-CoV-2[70].

### Sensitivity enhancement based on sample enrichment

Unlike nucleic acid, protein can not achieve quantity increasing by amplification. Fortunately, enrichment strategies, such as isoelectric electrophoresis, dialysis, and magnetic enrichment, can be integrated with protein detection to increase the concentration and enhance the detection sensitivity.

### Sensitivity enhancement based on electrophoresis

Paper-based ion concentration polarization (ICP) preconcentrators were integrated with LFA by Kim et al. [71] to enhance the detecting sensitivity of  $\beta$ -human chorionic



**Fig. 2** Sensitivity enhancement based on sample enrichment. **A** Paper-based ICP pre-concentrators integrated with LFA for  $\beta$ -hCG detection [71]. **B** Experimental setup for (a) ITP-enhanced LFA and (b) conventional LFA. (c) Experimental snapshots were taken at 5 different time points [72]. **C** The 3-D paper is well combined with ATPS and LFA for transferrin detection [73]. (a) The 3-D paper well device

was combined with a transferrin competition assay on nitrocellulose paper. Samples containing no transferrin were correctly diagnosed when using the (b) 1:1 or (c) 9:1 volume ratio of ATPS solutions. **D** Schematic of the integrated device of the semi-permeable membrane, glass fiber, and PEG buffer into with LFA [74]

gonadotropin ( $\beta$ -hCG) (Fig. 2A). Through a simple 9 V battery and low power consumption (about 81  $\mu$ W), a pre-concentration factor of 25-folds was obtained, and the detection sensitivity was enhanced by 2.69 times compared with that of the commercial LFA for  $\beta$ -hCG.

Isotachopheresis (ITP) was used by Moghadam et al. [72] to focus target analytes into a thin band and then was transported to the test line of LFA, resulting in a dramatic increase in the surface reaction rate and equilibrium binding (Fig. 2B). This strategy can improve the sensitivity of LFA by 400-fold compared with that of the commercial LFAs for  $\beta$ -hCG.

### Sensitivity enhancement based on extraction

Kamei's group [53] firstly developed an aqueous two-phase system (ATPS) to concentrate a target biomarker into a smaller volume before loading it onto LFA. Using this method, a tenfold improvement in the overall detection sensitivity of LFA was achieved for bacteriophage M13 [75] and transferrin (Tf) [76]. In addition, this newly discovered

concentrating phenomenon suggested that the paper membrane sped up the macroscopic phase separation of ATPS.

Chiu et al. [73] expanded the paper device vertically, thereby increasing the cross-sectional area of flow and exploiting the effects of gravity on macroscopic separation (Fig. 2C). In addition to accelerating phase separation, this 3-D component also enhanced the ability to process larger and more diluted volumes of sample. The novel integration of ATPS and LFA within a 3-D paper well successfully yielded a tenfold improvement in the detection of transferrin.

### Sensitivity enhancement based on dialysis

Tang et al. [74] integrated the semi-permeable membrane, glass fiber, and PEG buffer with LFA for samples enrichment (Fig. 2D). PEG-loaded glass fiber was used as the dialysate for sample concentrating. Compared with that of the conventional LFA, tenfold signal enhancement in HIV detection and fourfold signal enhancement in myoglobin detection have been achieved, respectively.

### Sensitivity enhancement based on magnetic enrichment

Taking advantage of supermagnetism, magnetic bead-based separation is used as a convenient way to perform sample pretreatment and eliminate the interference of food matrices in LFA. Generally, aptamers [77, 78] or antibodies [79, 80] are labeled with magnetic beads for target capturing.

Besides the capability of fast separation in the magnetic field, magnetic nanoparticles or magnetic beads possess the color of brown and can provide low background noise, which make them ideal label materials of LFA [6]. Zhang et al. [81] developed a multiple immunoassay test strip based on  $\text{Fe}_3\text{O}_4$  superparamagnetic nanosphere (SPMN) probes, which was used to enrich samples and quench the fluorescence of multiple fluorescer on the test line (Fig. 3). Simultaneous detection of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA153) was realized with LOD of 0.06 ng/mL and 0.6 U/mL, respectively.

### Sensitivity enhancement based on the change of flow rate

Another critical factor affecting the sensitivity of LFA is the time for the immunoreaction between the analyte and the capture antibody pre-deposited on the test line, which depends on the migration time of the sample over the test line [82]. The decrease in the reaction time causes insufficient time for the antibody-antigen reaction [83], so a test should be long enough for a sufficient antibody-antigen

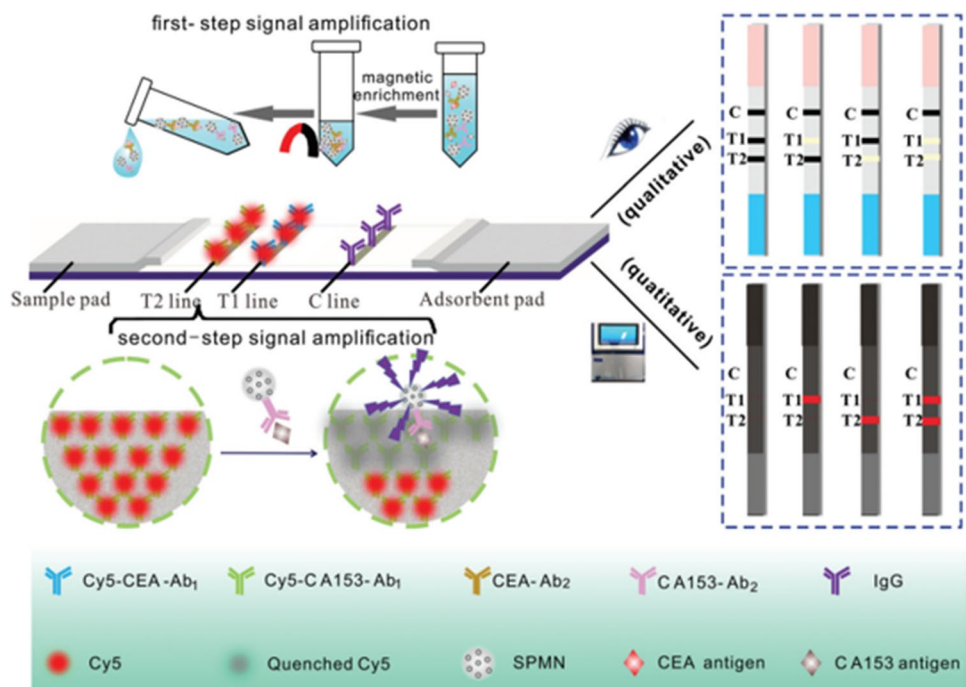
reaction. An increase in the distance from the conjunction pad to the test line can increase the reaction time [84]. An increase in the pore size of a nitrocellulose membrane can decrease the amount of bound protein and increase the flow rate. And, the rapid migration times caused a high signal-noise ratio [85]. More importantly, some engineering methods, such as flow block and NC membrane size change, have been developed for LFA sensitivity enhancement.

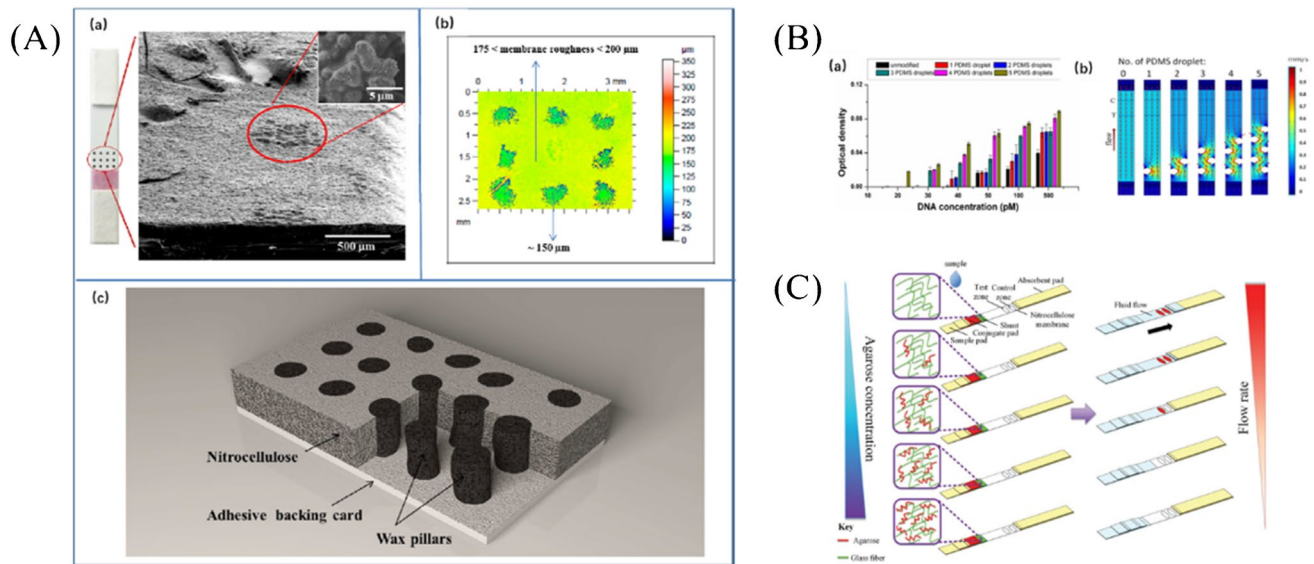
### Sensitivity enhancement based on flow block

Rivas et al. [86] developed delay hydrophobic barriers fabricated by wax printing to improve LFA sensitivity (Fig. 4A). When running buffer flows through the wax barrier, microfluidics delay and pseudo-turbulent flow were generated in the columnar region, which improved the sensitivity of almost 3-folds in comparison to a commercial barrier-free LFA.

However, the wax barrier on the NC membrane of LFA might melt during the process of heating [89]. A piece of paper-based shunt and a polydimethylsiloxane (PDMS) barrier were integrated with LFA by Choi et al. [87] to achieve optimal fluidic delays, and tenfold signal enhancement was obtained for detecting hepatitis B virus (HBV) nucleic acid (Fig. 4B). In further studies, Choi et al. [88] used agarose as the barrier due to its strong permeability, excellent mechanical properties, easier fluid control, and tunable pore size and porosity (Fig. 4C). Remarkably, this detecting strategy enhanced the sensitivity nearly 10-folds with a detection

**Fig. 3** Mechanism of magnetic fluorescence-based LFA platform for CEA and CA153 detection [81]





**Fig. 4** Sensitivity enhancement based on flow block. **A** (a) SEM image for the transversal cut of wax pillars area on LFA. (b) Surface profile roughness of LFA modified with wax pillars. (c) Schematic of a transversal cut of pillars zone on nitrocellulose membrane

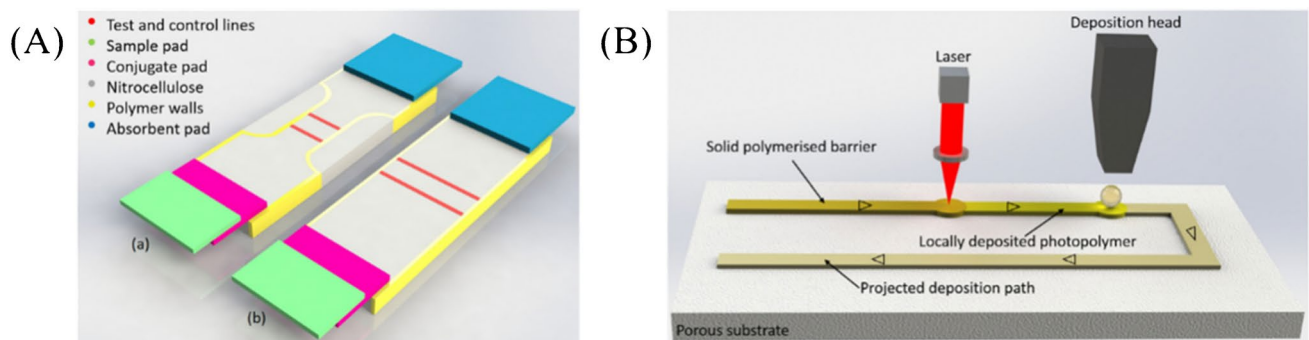
[86]. **B** Sensitivity enhancement in LFA by creating a PDMS barrier. (a) Effects of PDMS droplets numbers on LOD. (b) Results of flow velocity simulation of LFA [87]. **C** Schematic diagram of fluidic control in a paper-agarose hybrid material-based LFA [88]

limit of 100 copies of dengue viral RNA, which yielded comparable or more sensitive result than that of the published techniques of enzyme enhancement [90], temperature and humidity control [91], and fluidic control by hydrophobic barriers [86].

### Sensitivity enhancement based on NC membrane size change

Katis et al. [92] used spatial constrictions in the flow path as a route to increase the sensitivity and lower the LOD of LFA, due to the slower flow rate and the smaller test zone area (Fig. 5A). The liquid photopolymer was locally deposited onto the paper substrate with a deposition nozzle. A laser beam subsequently followed the deposition head,

illuminated the deposited patterns, and induced the photopolymerization of the polymer (Fig. 5B). The polymerized patterns defined the fluidic walls, which served as demarcation barriers to confine and transport the liquids within the paper device. The LOD of C-reactive protein (CRP) was 5 ng/mL with a 1-mm-wide constriction, which was a 30-fold enhancement compared with that of the standard LFA with the width of 5 mm.



**Fig. 5** Sensitivity enhancement based on NC membrane size change [92]. **A** Schematic of the flow path constriction device (a) and a standard lateral flow device (b). **B** Schematic of the modified laser-based direct-write procedure

## Sensitivity enhancement based on label evolution

### Sensitivity enhancement based on colorimetric methods

#### Sensitivity enhancement based on dual AuNPs

With an increase in the particle size of AuNPs, the molar extinction coefficient is significantly enhanced [93, 94]. Therefore, a larger particle size of AuNPs as the labeling nanomaterial is more conducive to improve the sensitivity [95].

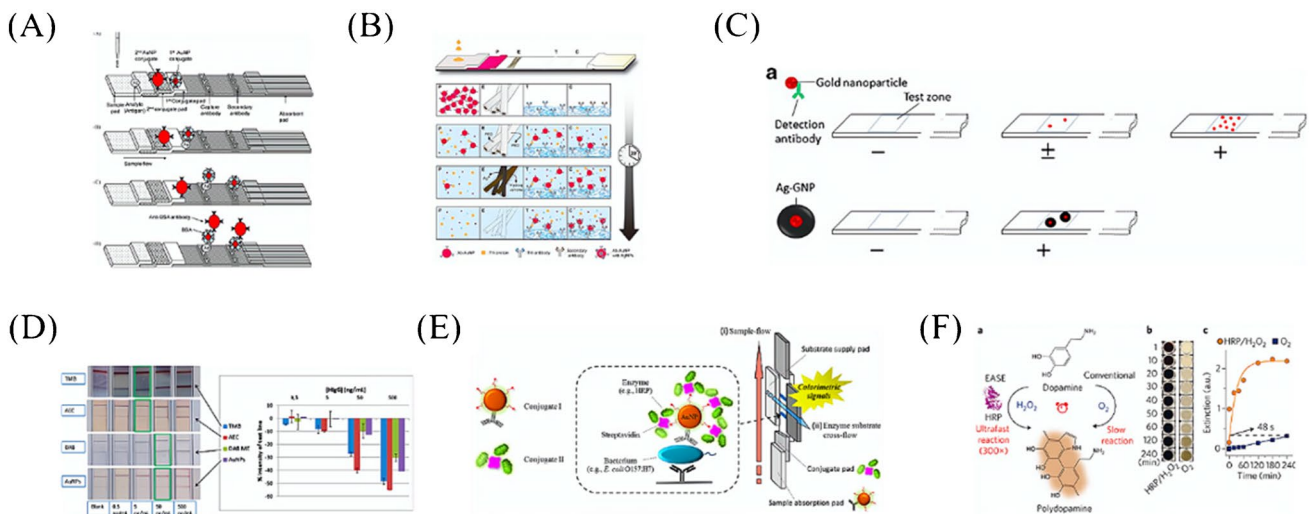
Choi et al. [96] used two different sizes of AuNPs to realize the sensitive detection of cardiac troponin I (cTnI) by LFA (Fig. 6A). The first conjugate was AuNPs (10 nm) coated with anti-troponin I antibody and blocked with bovine serum albumin (BSA), and the second conjugate was AuNPs (40 nm) coated with anti-BSA antibody and blocked with human serum albumin. Double AuNP-based LFA can detect as low as 0.01 ng/mL of cTnI within 10 min, which was enhanced about 100 times compared with that of Posthuma-Trumpie’ research [97]. This dual-labeling strategy was subsequently optimized for the on-site and sensitive detection of melamine in milk, offering a 10- to 25-fold improvement [98]. Later, this method was used in detecting bisphenol A (BPA) [99], Hg<sup>2+</sup> [100], and procalcitonin (PCT) [101].

### Sensitivity enhancement based on silver staining

Inspired by GNSs, the method of silver staining was developed to change the shape of AuNPs.

Silver staining is another method for signal amplification via the color change at the test line based on chemical reactions [106]. These reactions involve the catalytic reduction of silver ions on gold nanoparticles, which produces larger absorbance values for gold nanoparticles and darker colors at the test lines of LFA [107].

Yang et al. [108] used silver staining in LFA for the first time. When a visible red color appeared on the detection area, the NC membrane was covered with an AgNO<sub>3</sub> pad, and on which the reducer pad was placed. The produced larger size of silver particles was used to change the color of the detection line from red to black which made the contrast more obvious. A 100-fold improvement in sensitivity with a detection limit of 0.1 ng/mL for abrin-a was obtained. Kim et al. [102] used core-shell hybrid nanofibers and silver staining technique to detect cTnI. AgNO<sub>3</sub> and silver-reducing reagent of hydroquinone solutions were separately encapsulated by electrospinning (Fig. 6C). The silver ions were reduced to metallic silver around AuNPs and the color of the test line was darkened. The obtained detection limit for cTnI was 0.24 ng/mL, and the sensitivity was enhanced by up to 10 times compared with that of the commercial LFA. Anfossi et al. [103] used this method to realize the detection of ochratoxin A (OTA) by competitive LFA (Fig. 6D), and the sensitivity was enhanced by 10



**Fig. 6** Sensitivity enhancement based on colorimetric methods. **A** Dual AuNPs LFA was used for cTnI detection; the sizes of AuNPs were 10 nm and 40 nm, respectively [96]. **B** Schematic of a hybrid nanofiber-deposited LFA kit and the time-dependent changes of the conjugate pad (P), electrospun nanofibers E, test line (T), and control line C during the signal-enhanced assay [102]. **C** Expected effects of silver staining on LFA sensitivity enhancement [103]. **D** (Left)

Results of LFA for different concentrations of human IgG and the different substrates. (Right) Results were obtained with the strip reader [90]. **E** Schematic of AuNP-assisted signal amplification on LFA for pathogen detection [104]. **F** (a) Schematic of EASE. (b) The relationship between dopamine accumulation and time in the presence of HRP [105]

times compared with that of the traditional LFA for OTA detection [109].

### Sensitivity enhancement based on enzymatic amplification

Inspired by the wide use of enzymes as labels in bioassays, the nano-carrier-enzyme probes have been used in LFA to improve the detecting performance of various optical and electronic biosensing systems [110–113].

In the study of Parolo et al. [90], AuNPs were used not only as labels for antibodies, but also as carriers for enzymes (Fig. 6E). When acting as direct labels, AuNPs turned red at the test line and the control line of LFA. However, after AuNPs conjugating with antibody and blocking with horseradish peroxidase (HRP), the produced insoluble chromogens cannot move by the flow and then darken the color of the lines. By comparing three substrates (TMB for blue-violet, AEC for red [114], and DAB for gray-black [115]), it was found that TMB is optimal for signal enhancement maintaining at an order of magnitude.

Cho et al. [104] used the abovementioned signal amplification technique for *E. coli* O157:H7 detection (Fig. 6F). The obtained detection limit was 100 CFU/mL, which was about 1000 times lower than that of the traditional AuNP-based LFA.

Li et al. [105] developed a universal “add-on” technique called enzyme-accelerated signal enhancement (EASE) (Fig. 6G). EASE depended on the ultrafast and localized deposition of polydopamine (PDA) at the test line [94], permitting a large number of signal molecules to be captured and leading to sensitivity enhancement over three orders of magnitude. Under the normal conditions of using DAB as the substrate, p24 at the concentration of 10 ng/mL can be detected by LFA, while by EASE, the detection limit can be obtained at the level of 10 pg/mL, allowing the ultrasensitive detection of HIV antigens with naked eyes.

### Sensitivity enhancement based on surface-enhanced Raman scattering (SERS)

In the excitation region of some specially prepared metal conductor surfaces or solutions, a stronger Raman signal caused by surface roughness can be observed, which is called surface-enhanced Raman scattering (SERS). Compared with traditional Raman scattering, SERS can realize 6 orders of magnitude or more [116].

Covian et al. [117] developed SERS-based LFA to detect pneumolysin by using gold-core-silver-shell nanoparticles as the plasmonic platform and rhodamine B isothiocyanate as Raman tag. Compared with the results of the electrochemical immunosensor with the detection limit of 0.6 ng/mL [118] and the chemiluminescence immunoassay with the detection limit of 5.5 pg/mL [119], the sensitivity of

SERS-based LFA was enhanced with the detection limit of 1 pg/mL.

It has been proved that SERS nanotags have the coding capacity by absorbing different Raman dyes on the surface of the metal [120], which made it possible to realize multiple detections. Zhang et al. [121] encapsulated Raman dye Nile Blue A (NBA) in the interface of the core–shell structure (Fig. 7A), and based on the core–shell SERS nanotags, a novel LFA was developed to realize rapid quantification of creatine kinase isoenzyme (CK-MB), cTnI, and myoglobin (Myo) on three test lines with a detection limit of 0.55, 0.44, and 3.2 pg/mL, respectively, which decreased the LOD nearly three orders of magnitude than that of the colorimetric detection [122, 123]. In the follow-up study, Zhang et al. [124] encapsulated Raman dyes methylene blue (MB), Nile blue A (NBA), and rhodamine 6G (R6G) in the core–shell interface to achieve rapid quantification of three cardiac biomarkers on a single test line with the similar detection limit (Fig. 7B).

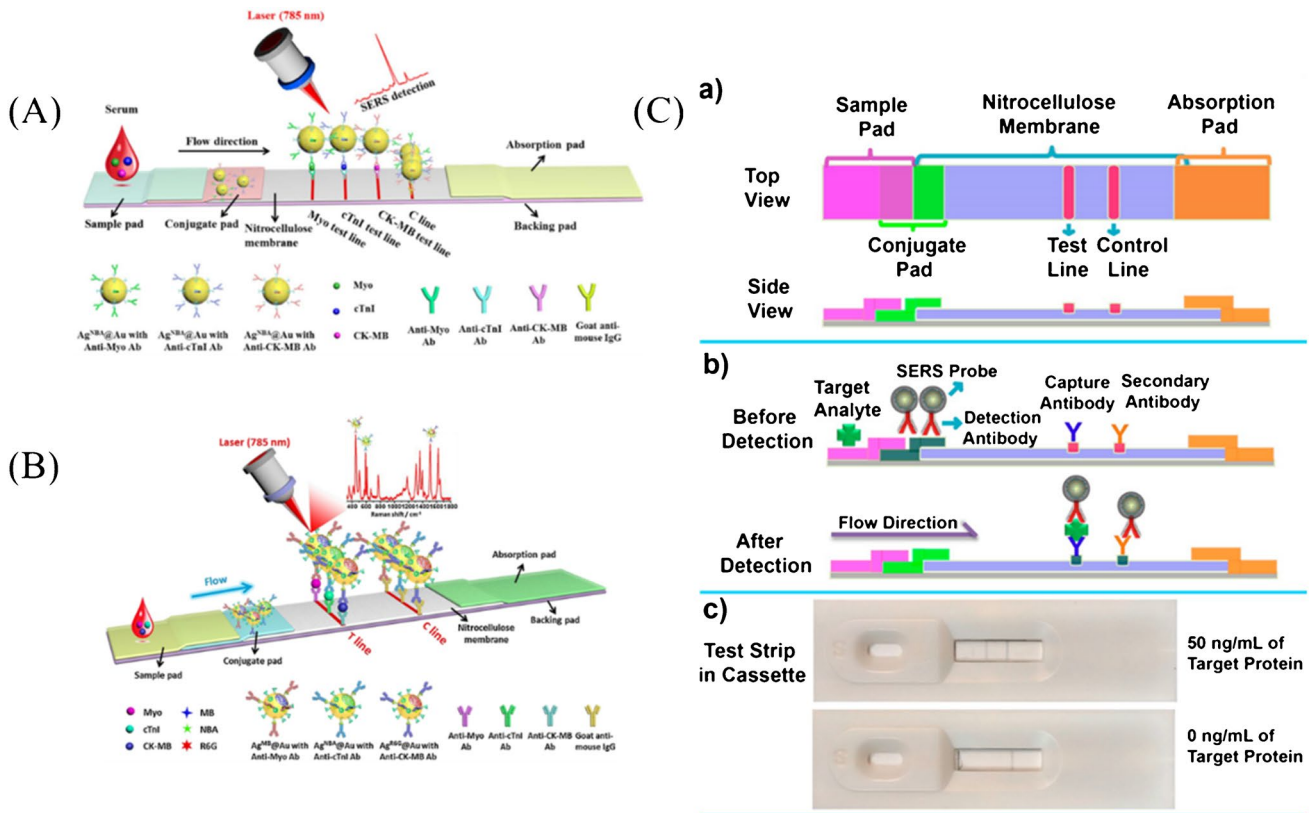
The stability of Raman dye plays a pivotal role in sensitive and accurate detection. Gao et al. [126] developed Au nanostar @ Raman dye @ silica sandwich nanoparticles for the detection of neuron-specific enolase (NSE), a marker of traumatic brain injury (TBI), with the LOD of 0.86 ng/mL. By wrapped between AuNPs and thin silica, Raman dyes malachite green isothiocyanate (MGITC) can be effectively stabilized. Compared with the traditional colorimetric method, the SERS-based method exhibited excellent performance especially in the matrix of plasma [127]. Hwang et al. [125] combined hollow gold nanospheres (HGN) with Raman dye MGITC to achieve the detection of staphylococcal enterotoxin B (SEB) (Fig. 7C). With the advantage of HGN enhancing SERS signal, high-sensitivity detection with a LOD of 1 pg/mL was realized, and more than 30 times enhancement was obtained compared with that of the traditional ELISA.

### Sensitivity enhancement based on photothermal methods

Li et al. [128] developed a quantitative photothermal-sensing LFA for enrofloxacin detection with the detection limit of 0.023 ng/mL (Fig. 8A). Black phosphorus (BP)-Au nanosheets showed good photothermal properties at the wavelength of 808 nm, and the photothermal conversion efficiency was enhanced by 12.9% compared with that of the black phosphorus nanosheets alone. Qin et al. [129] developed a thermal contrast-based technique to improve the sensitivity of LFA (Fig. 8B). The sensitivity can be enhanced by 32 times compared with that of the FDA-approved LFA for cryptococcal antigen (CrAg) detection.

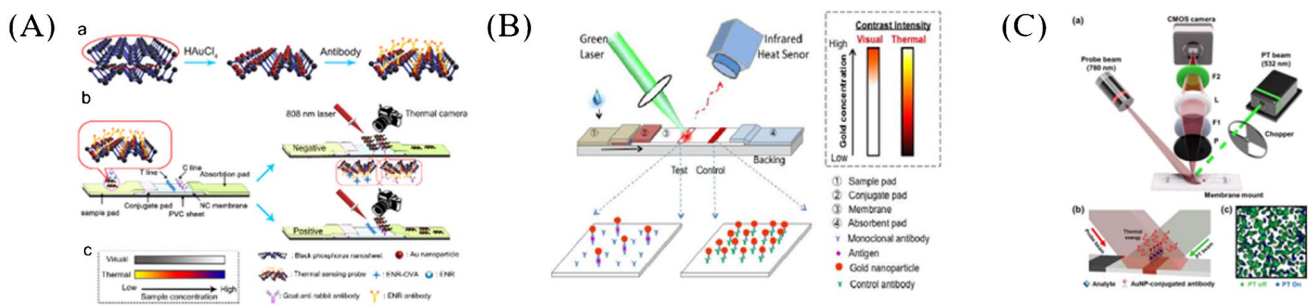
Wang et al. [131] developed a thermal contrast magnification (TCA) reader, which was composed of an emitter for





**Fig. 7** Sensitivity enhancement based on SERS. **A** Schematic illustration of the core-shell SERS nanotag-based multiplex LFA for three cardiac biomarkers detection [121]. **B** Schematic illustration of quantitative LFA for three cardiac biomarkers detection on a single T line with different roman dyes encoded core-shell SERS nanotags [124].

**C** Schematic illustration of the principle of SERS paper-based lateral flow strip (PLFS) based on HGN [125]. (a) Top and side views; (b) side view before and after detection of protein biomarker; (c) optical results of PLFS assembled in cassettes in the presence (upper) and absence (bottom) of the target protein



**Fig. 8** Sensitivity enhancement based on the photothermal method. **A** Schematic of PT-ICSSs [128]. (a) Preparation of BP-Au-Ab photo-thermal-sensing probe. (b) Structure and procedures of PT-ICSSs. (c) Comparison of colorimetric results with photothermal results. **B** Principle of thermal contrast for LFA [129]. **C** Schematic of the PT-LSI

sensor [130]. (a) A 780-nm light illuminated the NC membrane of LFA. (b) The absorbed light energy was converted into heat, and the resultant temperature increase altered the speckle pattern. (c) Exemplary speckle images before (green) and after (blue) photothermal illumination

lasers of multiple wavelengths emitting, an infrared camera for the generated heat reading, and software for data reading and analysis. The reader can significantly increase the accuracy of antigen quantification by LFA. Compared with naked eyes or colorimetric readers (such as BD Veritor™ system

readers), the TCA reader possessed a higher sensitivity of 8 times for detection of *malaria* and *C. difficile* [97, 132]. Laser speckle is the high-contrast random granular pattern, which is extremely sensitive to the refractive index and physical displacement of the medium [133, 134]. NC

membrane is composed of randomly oriented nanofibers, so it can be used as a diffusion medium to generate high-contrast speckle patterns. Song et al. [130] developed a photothermal laser speckle imaging (PT-LSI)-based LFA (Fig. 8c). The sensitivity can be enhanced by 68 times compared with that of the FDA-approved LFA for CrAg detection. The detecting ability of the developed PT-LSILFA was verified for CrAg detection by US FDA-approved LFA, and it was found that the detection sensitivity of PT-LSI was enhanced by 68 times.

All the parameters of the abovementioned LFA for rapid detection are compared and summarized in Table 1.

## Conclusions and future perspectives

LFA has been proven to be a rapid, sensitive, and cost-effective method for point-of-care and in-field diagnosis in resource-limited areas such as developing countries and rural areas. However, there are long-standing criticisms of LFA as POCT, such as limited sensitivity, limited ability

for quantification, inability for multistep performing, and inability to multiplexing.

In this paper, recent development and breakthroughs of the sensitivity enhancement for LFA are reviewed, such as sample pretreatment, changes in structure, materials, and labels, with an objective evaluation. For sample pretreatment, various isothermal nucleic acid amplification techniques, such as RCA, LAMP, and RPA, have been used in LFA, which makes the detection more sensitive. However, there are still challenges associated with the integration of amplification and detection into a single device. In addition, the reproducibility of complex enzymatic reactions should be considered. Changing the flow rate to enhance sensitivity through the flow barrier extends the time for antibody-antigen reaction; however, structural changes increase the difficulty of engineering and undoubtedly limit the application of LFA to some extent. AuNPs are the most popular labels for LFA. AuNPs integrated with other materials such as silver, gold, enzymes, or catalytic metals can further enhance the sensitivity of LFA, but this strategy has limitations regarding the preparation, purification, storage, and detection steps.

**Table 1** Comparison of sensitivity enhancing effect of LFA based on different principles

Method	Labels	LOD	Promotion degree	Advantages	Disadvantages	References
Isothermal nucleic acid amplification based	AuNPs	Single copy or lower 10 copies	/	Sensitivity	Enzyme inactivation	[38, 135]
Electrophoresis based	AuNPs	ng or pg/mL level	One or two order of magnitude	Without substances interference	Equipment dependent	[71, 72]
Extraction based	AuNPs	ng or pg/mL	An order of magnitude	Without substances interference	Non-portable	[73, 75, 76]
Dialysis based	AuNPs	ng or pg/mL level	An order of magnitude	Without substances interference	Equipment dependent	[74]
Magnetic enrichment based	AuNPs	ng or pg/mL level	An order of magnitude	Without substances interference and easy to separate	Expensive for functional magnet based	[136, 137]
Dual AuNPs based	AuNPs	ng or pg/mL level	An order of magnitude	Low cost, rapidness, easy to operation, and naked-eye readout	Disability to quantification, low sensitivity	[96, 98–101]
Silver staining based	AuNPs, AgNPs	ng or pg/mL level	An order of magnitude	Low cost, rapidness, and naked-eye readout	Multiple steps, low sensitivity	[102, 103, 108]
Enzymatic amplification based	AuNPs	ng or pg/mL level	An order of magnitude	Low cost, rapidness, and naked-eye readout	Multiple steps, enzyme inactivation	[90, 104, 105]
SERS based	MGITC, rhodamine B, rhodamine 6 G Nile blue A, methylene blue	Close to fg/mL level	3–4 orders of magnitude	Sensitivity, rapidness, and quantification	Equipment dependent	[125, 138, 139]
Photothermal illumination based	AuNPs	Close to fg/mL level	3–4 orders of magnitude	Sensitivity, rapidness, and quantification	Equipment dependent	[128–131]

Accordingly, the latest research indicates that there is still a large development space for LFA with smaller demand volume, shorter analysis time and the absence of hook effect, and higher accuracy and sensitivity. Here, several possible directions for sensitivity enhancement of LFA are summarized as follows.

Firstly, the development of new materials, including new labels, probes, and paper-based materials. The super-strong signal characteristics of aggregation-induced emission (AIE) molecules in the solid phase are promising to become an excellent label for LFA. Novel capturing/detecting probes, such as nanobodies, short peptides, and aptamers, are also being developed to enhance stability, to increase detection sensitivity, and to minimize the cross reaction. In addition, with the smaller molecular weight, they can be used to target small molecules or epitopes that are inaccessible for conventional antibodies.

Secondly, for the detection of nucleic acid, a certain nucleic acid amplification method is currently used for sample pretreatment. However, a single nucleic acid amplification method may not satisfy the detecting requirements. Therefore, a cascade amplification detection method that uses two or even multiple nucleic acid amplification methods integrated with LFA will be great development potential.

Finally, miniaturization and optimization of LFA devices are another vital goal for the sensitivity enhancement of LFA. It must be noted that among LFA detection methods, optical methods based on AuNPs and their derivatives are most likely to be one of the most viable POCT devices, given their simplicity and easy integration in conventional LFA. Therefore, significant engineering efforts should be focused on miniaturizing the whole process to be used in portable devices, which will be affordable and portable in field conditions and resource-limited settings.

**Acknowledgements** This work was financially supported by the Space Medical Experiment Project of China Manned Space Program (HYZHXM04003).

## Declarations

**Conflict of interest** The authors declare no competing interests.

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