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New Technologies and Reagents in Lateral Flow Assay (LFA) Designs for Enhancing Accuracy and Sensitivity

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Abstract

Lateral flow assays (LFAs) are a popular method for quick and affordable diagnostic testing because they are easy to use, portable, and user-friendly. However, LFAs design has always faced challenges regarding sensitivity, accuracy, and complexity of the operation. By integrating new technologies and reagents, the sensitivity and accuracy of LFAs can be improved while minimizing the complexity and potential for false positives. Surface Enhanced Raman Spectroscopy (SERS), Photoacoustic techniques, Fluorescence Resonance Energy Transfer (FRET), and the integration of smartphones and thermal readers can improve LFA accuracy and sensitivity. To ensure reliable and accurate results, careful assay design and validation, appropriate controls, and optimization of assay conditions are necessary. Continued innovation in LFAs technology is crucial to improving the reliability and accuracy of rapid diagnostic testing and expanding its applications to various areas, such as food testing, water quality monitoring, and environmental testing.

Keywords: Lateral flow assay; virus detection; bacteria detection; point-of-care; COVID-19; biosensors.

1. Introduction

The demand for quick tests is growing, encouraging the creation of novel methods for agriculture, human and animal health diagnosis, environmental monitoring, food analysis, military, and forensic science [1]. Therefore, creating affordable, reliable, and quick diagnostic testing equipment for use outside laboratories is crucial. The availability of accurate point-of-care (POC) diagnostic tools for just four infective diseases—malaria, bacterial pneumonia, syphilis, and tuberculosis—is thought to be able to avert at least 1.2 million deaths annually worldwide[2-4]. The point of care (POC) test is commonly known as the lateral flow assay (LFA), alternatively

referred to as a lateral flow device (LFD), lateral flow test (LFT), or quick test. This method was first reported in 1956 [3] and is a straightforward technique designed to identify a target material in a liquid sample without sophisticated or expensive equipment. In the early 1970s, the LFA was first commercially developed as a home-use pregnancy test to analyze human chorionic gonadotropin (hCG) hormone in urine samples [5].

The assay is typically formed by sandwiching [6] a sample between two material layers, a test strip, and a conjugate pad (**Figure 1**). The test strip is typically made of a porous material, such as nitrocellulose or cellulose acetate, that allows the sample to flow through it. The strip contains a test line, where a colored line appears if the analyte is present in the sample. The test line is made of a material that binds specifically to the analyte of interest, such as an antibody or a protein. The conjugate pad includes a second reagent that binds precisely to the target analyte. The conjugate pad is often made of a non-porous substance that prevents the flow of the sample, like cellulose or polyester. Usually, a tracer, such as gold nanoparticles, an enzyme, or a fluorescent dye, is added to the conjugate pad to attach to the analyte and give an indication that can be seen. The sample runs through the test strip after being applied to it, and then it bonds to the test line. The second reagent is subsequently bound to the sample as it passes through the conjugation pad. If the analyte is present in the sample, a colored line will appear on the test strip, indicating a positive result. A control line should appear for both positive and negative results to check the test's validity and verify the device's functioning. The sensitivity and specificity of the assay can be fine-tuned by using different antibodies, proteins, or other reagents, which bind specifically to different regions of the analyte.

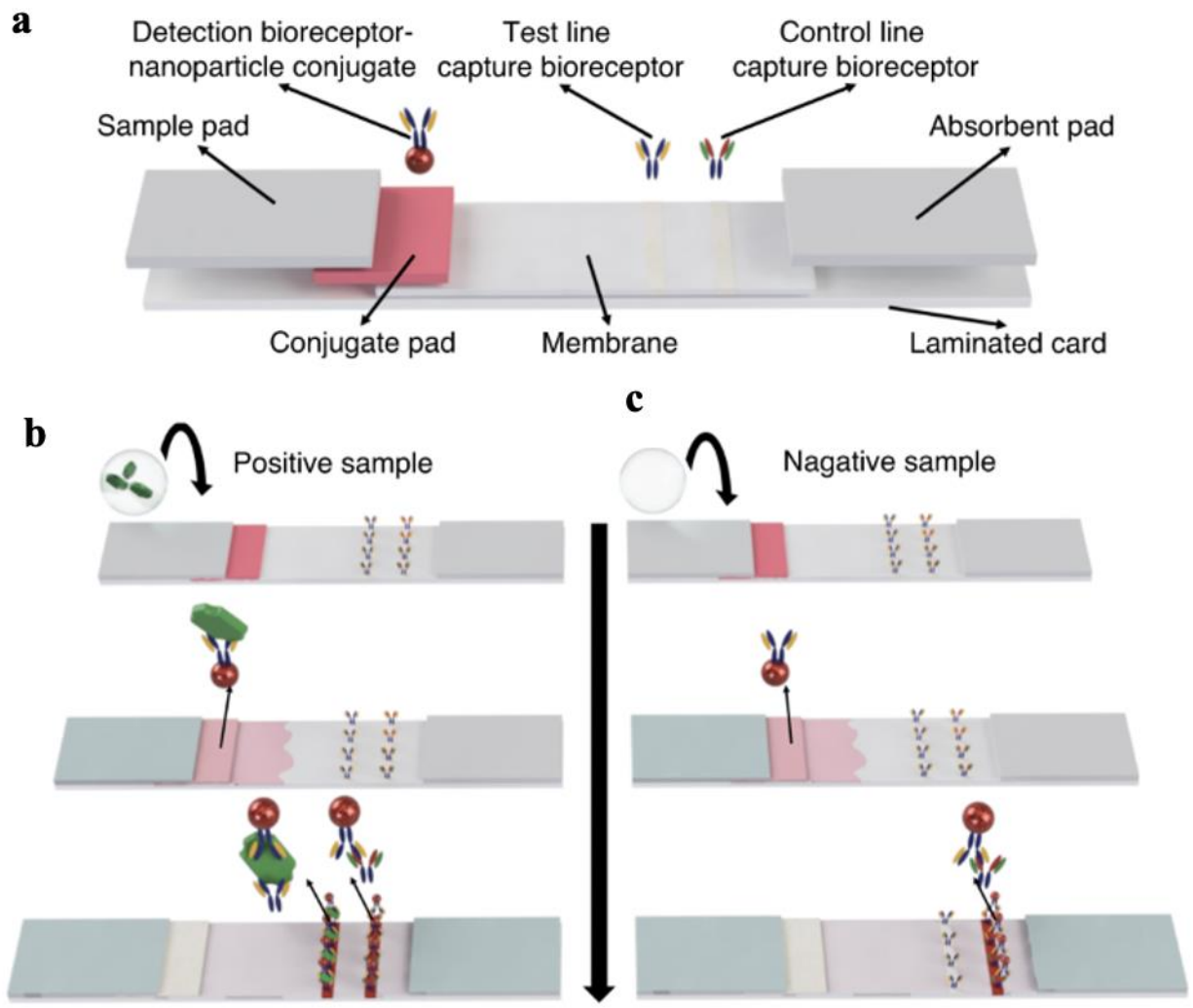

Figure 1. Schematic showing the essential parts and working principle of an LFA device. (a) The main components of LFA devices are the absorbent pad, conjugate pad, membrane, and sample pad. All these parts are laminated on a card. An example of LFAs based on immunosandwich recognition is demonstrated in Panels b and c. (b) When the target analyte is available in the sample, nanoparticles build up on both the control and test lines; (c) when the target analyte is absent in the sample, the nanoparticles exclusively accumulate on the control line. [7].

The lateral flow assay (LFA) design has evolved, with different reagents being used to detect specific analytes. Some of the most common trends in LFA design include the use of antibodies, enzymes, peptides, mRNAs, fluorescence, and quantum dots. Antibodies are proteins that bind to a specific target, such as a virus or disease marker [8]. Enzymes are proteins that catalyze chemical reactions and are commonly used in LFAs. For example, the enzyme replaces

glucose with gluconic acid and hydrogen peroxide in glucose testing, generating a visible signal [9]. Peptides are short chains of amino acids used in LFAs as the test line reagent [10]. Messenger RNA (mRNA) is a genetic material that carries genetic information from DNA to the ribosome [11]. Fluorescence is the property of some molecules to release light when excited by the specific wavelength of light. Fluorophores are often used as tracers in LFAs [12] to generate a visible signal. Quantum dots are tiny semiconductor particles that can be used as tracers in LFAs[13] and are often used in LFAs due to their high fluorescence intensity, stability, and versatility. Finally, antibodies, enzymes, mRNA, and peptides are commonly used in LFAs as the test line reagent. They are often labeled with a tracer such as gold nanoparticles, enzymes, or fluorescent dyes to generate a visible signal. These are some common trends in LFAs design, but new technologies and reagents are constantly being developed.

This review presents the LFAs' new integration trends using Raman spectroscopy, Photoacoustic, fluorescence, smartphone, thermal reader, and other reagents. Integrating these advanced technologies and reagents with lateral flow assays can enhance the accuracy and sensitivity of the results, achieve faster results, accomplish more convenient sample preparation, and lead to cost savings. In addition, integrating these technologies and reagents has enabled the development of rapid POC diagnostics, which can be used in isolated and underdeveloped areas and traditional clinical settings.

2. LFA design and readout systems

2.1 Comparison of different LFAs designs

Many POC lateral flow assays are available, each with unique design features. The following table compares the features of five popular POC LFAs based on the limit of detection (LOD) ranges, analytical sensitivity, and detection times. It should be noted that the transformation of units between g/mL and molarity (M) can be done by calculating the molar weight of the analytes. The table compares the performance of traditional LFA devices with that of Electrochemical, Surface Enhanced Raman Scattering (SERS), Fluorescence, Photothermal, and Magnetic-based LFA devices. An electrochemical lateral flow assay (eLFA), which is mainly a biosensor, introduces an automated signal detection technique for improving analytical implementation in terms of facilitating sensitivity, rapidity, and selectivity [14]. Chemically enhanced LFAs (Laser-induced Fluorescence Assays) use new fluorescence-based labels and

reagents to improve specificity and accuracy [15]. Photothermal LFAs use photothermal laser speckle imaging, thermal contrast photoacoustic imaging, and thermal photonic lock-in imaging [16-19]. SERS coupled with PCR (Polymerase Chain Reaction) utilizes amplification of the analyte to detect single base changes in DNA [20]. Magnetic lateral flow immunoassays utilize magnetic nanoparticles (MNP) as the detection label instead of the traditional gold or latex beads to facilitate the enrichment of the analytes. These MNPs can be detected and measured using external devices, enabling the creation of immunochromatographic tests that can produce quantitative results [21].

Table 1. Comparison of different LFAs design

LFA Design	LOD (M)	Analytical sensitivity (μg/mL)	Time to detection (min)	Reference
Traditional	10^{-5}~ 10^{-7}	0.1	5 ~ 10	[22]
Electrochemical	10^{-9} ~ 10^{-15}	4.6×10^{-6}	10 ~ 60	[23]
SERS	10^{-9} ~ 10^{-15}	10^{-7}	15 ~ 30	[24]
Fluorescence	10^{-8} ~ 10^{-15}	0.008	10 ~ 60	[25]
Photothermal	10^{-7} ~ 10^{-13}	10^{-5}	10 ~ 25	[17]
Magnetic	10^{-7} ~ 10^{-13}	1.6×10^{-8}	10 ~ 30	[26]

2.2 Comparison of the readout system

Different readout systems are available for LFAs, including smartphones, upconverting nanoparticle (UCNP), surface-enhanced Raman spectroscopy (SERS), photoacoustic and thermal contrast reader systems. Each type of readout system has advantages and disadvantages that must be considered when selecting the appropriate one for a given application. Greater emphasis will be given to those readers best suited to commercialization based on comparing their price, dimensions, operation mode, and reported assay sensitivity, as shown in Table 2 [27].

Table 2. Comparison of the readout system

Readout system	Signal	Application	LOD (pg/mL)	Price ($)	Size (mm^3)	Weight	Ref
Smartphone	Colorimetric and luminescence	E. coli 0157:H7 HIgG, EVD-IgG, HIgG, PSA, AFB1	590 ~ 2×10^5	200 ~ 250	15× 8 ×1 ~ 136× 69 ×7	130 ~ 400	[28-33]
UCNP reader	luminescence	Triamcinolone acetonide,ST-2, ochratoxin A	980 ~ 5000	650 ~ 700	240× 94 ×54 ~ 300× 300 ×150	900 ~ 2000	[34-36]
SERS reader	SERS	hcG	106	3000	180× 110 ×47	1000	[37]
Photoacoustic reader	PA	glucose	5.4×10^6	1300	40× 20 ×20	4000	[38]
Thermal contrast reader	TCA	hcG	180	500	133× 108 ×73		[39]

3. New technologies, systems, and reagents used in LFAs design

3.1 Surface-enhanced Raman spectroscopy-based LFAs

Gold nanoparticles are commonly used in traditional colorimetric LFA (CLFA) because they are easy to make and can be seen with the naked eye. However, CLFA has low sensitivity compared to other methods, so it is not always suitable for measuring analytes with low concentrations. CLFAs are primarily used for Yes/No type binary decision-making applications (presence or absence of the analyte). To measure the analytes' concentration, the test zone's color can be scanned and analyzed with equipment or a smartphone app. However, this procedure has a restricted working range and is highly nonlinear. Therefore, there is a need to find new labels and readout technologies for LFA. SERS tags leverage the plasmon response of nanoparticles used in numerous analytical and imaging applications. They consist of Au or metal nanoparticles with

Raman-active molecules and recognizing antibodies. SERS tags are more efficient than fluorescent labels, and their Raman scattering intensity allows for detecting analytes with very low concentrations. Additionally, the narrow spectral bandwidth of Raman spectra is suitable for multiplexing, and studies have shown increased sensitivity and dynamic range in LFA using SERS readout modalities.

SERS-LFA has many advantages over traditional LFA, including a much lower LOD (Table 3) and the ability to detect a wide range of analytes quantitatively and multiplexed. However, the need for costly equipment and long signal processing time restricts its use as a first choice for POC applications. Recent developments in compact SERS readers for LFA strips show promise in solving these issues. Further developments could include creating a lateral flow strip with low fluorescence background and modifying SERS tags to eliminate nonspecific adsorption. The integration of SERS-LFA with automated biochips has the potential to create a compact and highly sensitive detection platform suitable for POC diagnostics.

Table 3. Comparison of LOD of tradional and SERS LFAs

Sample	LOD of Traditional LFA	LOD of SERS LFA	Reference
Troponin I	5 ng/mL	0.09 ng/mL	[40]
TSH	1.5 µIU/mL	0.025 µIU/mL	[41]
HIV	80 pg/mL	8 pg/mL	[42]
Staphylococcal enterotoxin B	10 ng/mL	0.001 ng/mL	[43]
H1N1	67 ng/mL	6.7 ng/mL	[44]
Influenza virus A	5×10^4 pfu/mL	1.9×10^4 pfu/mL	[45]
Zika	10 ng/mL	0.72 ng/mL	[46]
Dengue	50 ng/mL	7.67 ng/mL	[46]

SERS–Based Sandwich Immunoassay was used to detect Rabbit Immunoglobin G, significantly saving assay time without losing sensitivity [47]. A similar concept had been used for SERS-based LFAs assay to quantitatively test a human immunodeficiency virus type 1 (HIV-1) DNA in the low concentration range. This SERS-based lateral flow assay has a detection limit of 0.24 pg/mL, which was at least a thousand times more sensitive than colorimetric or fluorescent

detection techniques [48]. The schematic diagram of the arrangement and the operational principle of the SERS-based lateral flow assay is depicted in **Figure 2.**

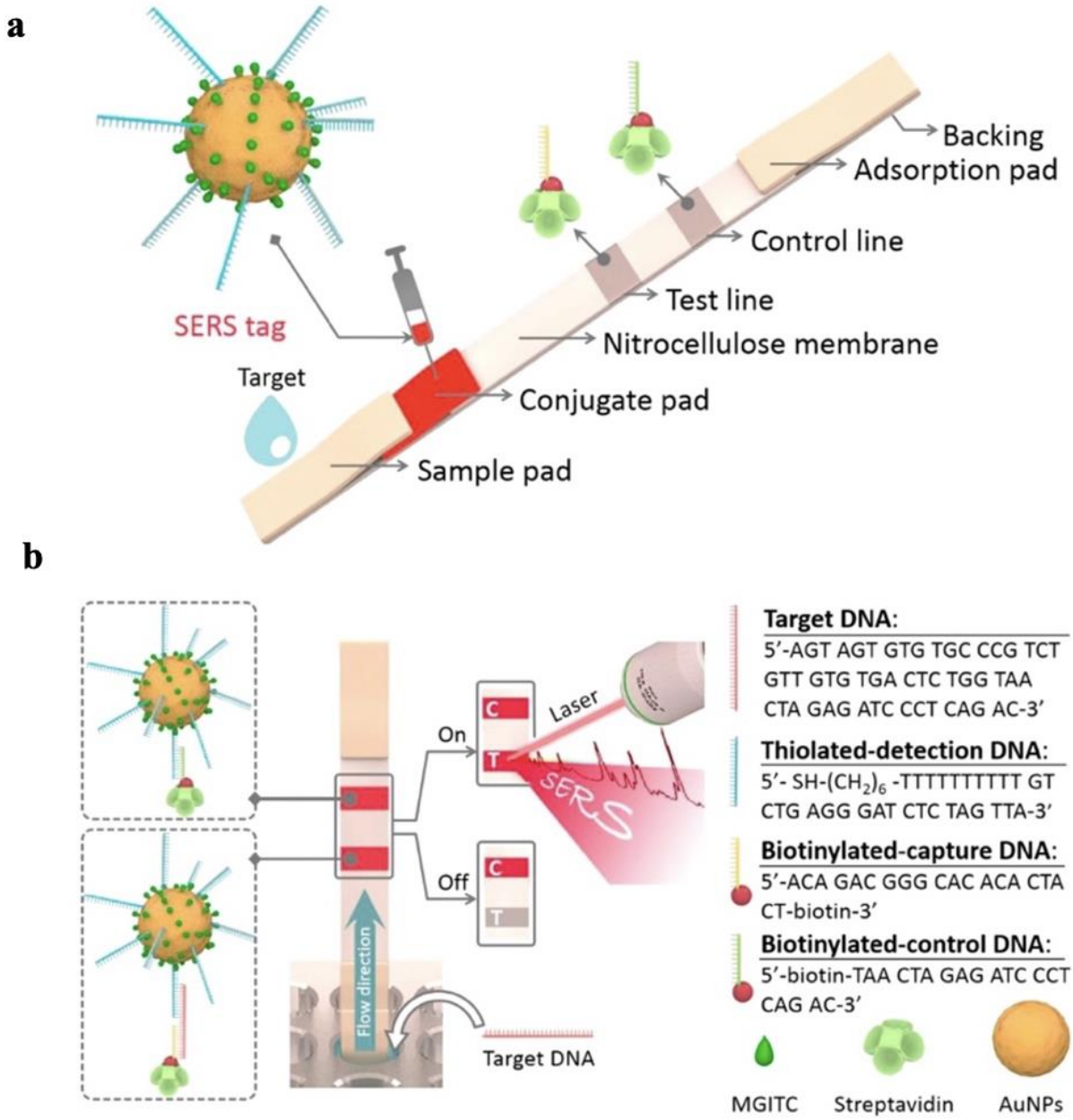

Figure 2. Schematic showing the (a) design and (b) working principle of SERS-based LFA device. Here, T = test line, and C = control line. [48].

3.2 Photoacoustic-based LFAs

Compared to traditional lateral flow assay devices, photoacoustic lateral flow assay devices have multiple benefits. Firstly, they are more sensitive and specific since they use a photoacoustic signal generated by laser absorption to detect small amounts of analyte with high precision. Secondly, the ability to detect numerous analytes simultaneously in one sample is feasible with photoacoustic-based LFAs. Thirdly, they have a wider dynamic range, allowing for accurate measurement of high and low analyte concentrations. Lastly, they are convenient and portable, perfect for point-of-care testing (POCT) in distant or resource-constrained environments.

The pioneering work of Zhao et al. [17] introduced the concept of a photoacoustic-based lateral flow test, showcasing the quantitative identification of a disease biomarker by leveraging the strong interaction between light and gold nanoparticles. The history of photoacoustic point-of-care testing dates to the 1970s [49], when the first prototype devices were developed. These devices were used to measure blood oxygen saturation in a non-invasive approach. Since then, photoacoustic point-of-care testing has evolved to include a variety of measurements, including glucose, cholesterol, uric acid, and other biomarkers. Compared to colorimetric measurements, photoacoustic analysis improved the detection limit of a commercially available lateral flow test strip by two orders of magnitude [17]. **Figure 3** shows the typical design of photoacoustic-based LFA and two different arrangements of PA detector setups. The test strip includes nanoparticles that are bound to the target analyte. A photoacoustic sensor detects the sound waves produced when light is illuminated on the test strip and after the nanoparticles have absorbed the light. This creates a signal showing the analyte's presence in the sample. This kind of test has the potential to be extremely sensitive, precise, and quick, making it helpful in several contexts, including remote or underdeveloped places. Its integration with existing diagnostic platforms makes the potential to transition from laboratory research to preclinical and clinical reality possible [50].

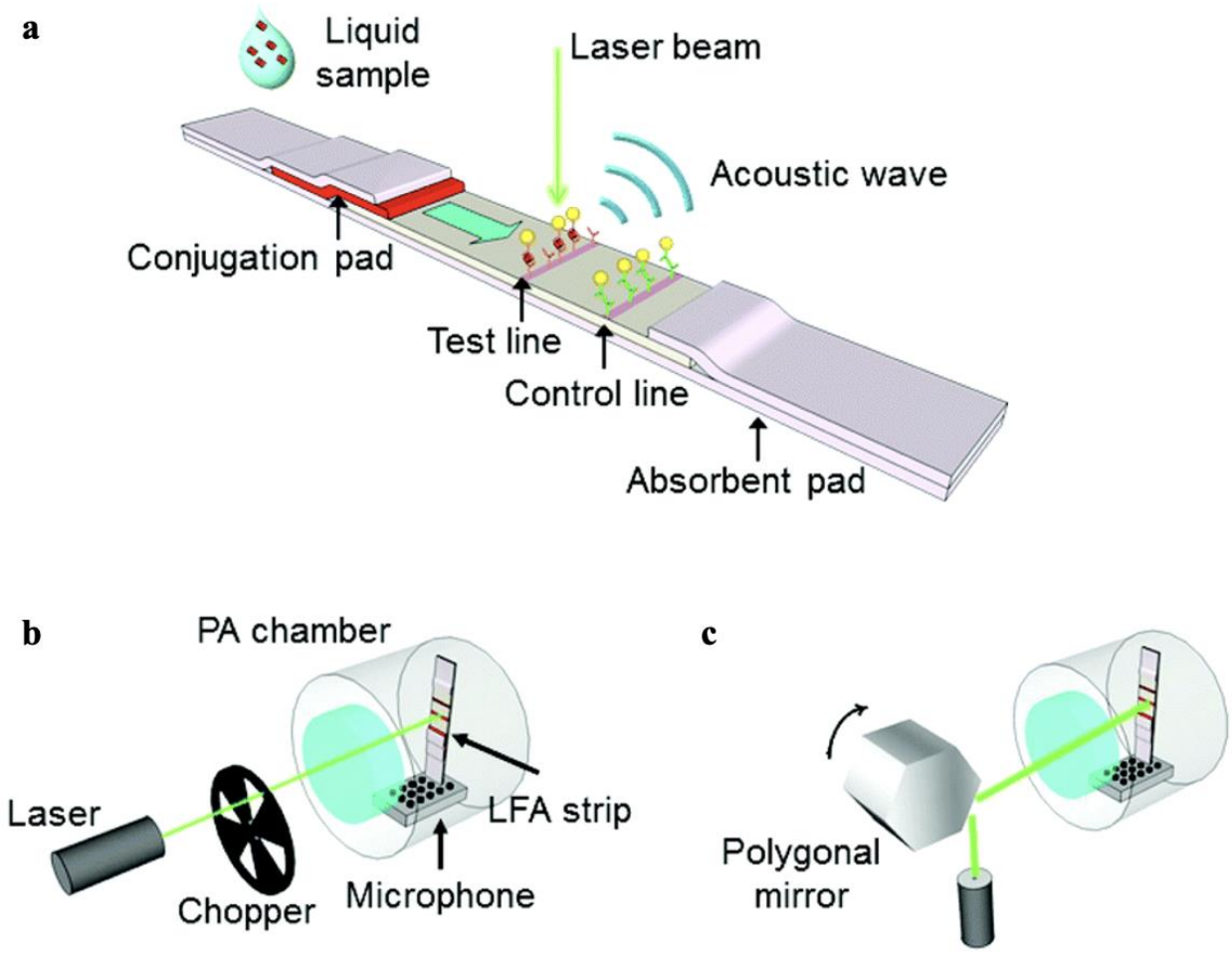

Figure 3. Schematics of two different arrangements of PA detection setups. (a) Illustration of laser beam producing PA signals on an LFA device. The acoustic signal can be detected using a simple microphone. Schematic of (b) chopper-based and (c) scanner-based PA detection systems [17].

3.3 Fluorescence Resonance Energy Transfer (FRET) Based LFAs

Compared to traditional LFAs, FRET-based LFAs have several benefits. They offer higher sensitivity and specificity and can be used for multiplex detection and real-time monitoring of analyte binding. They also have a more comprehensive dynamic range and can be easily automated for high-throughput analysis. These advantages are due to the FRET signal, generated only when the target analyte is present, reducing false positives. Further, FRET-based LFA could be used in complex sample matrices, including blood, saliva, serum, and urine, making them useful for various applications.

Wang et al.[51] developed a high-performance fluorescence LFA strip (**Figure 4**) by using S protein-conjugated dual quantum dots (SiO_2@DQD) nanotags for the detection of SARS-CoV-2-specific antigens (IgM/IgG) in clinical samples. The synthesized NPs had excellent luminescence, monodispersed, and good stability properties. The anti-human IgM and IgG antibodies were immobilized on two test lines to simultaneously and accurately identify anti-SARS-CoV-2 IgM and IgG in a single human sample. They achieved this by immobilizing the SARS-CoV-2 spike protein onto the surfaces of SiO2@DQD NPs. The proposed SiO2@DQD-based LFA strip demonstrated the ability to rapidly and precisely detect low concentrations of IgM/IgG (at a dilution of 1:107) from just one μL of serum in only 15 minutes. Similar approaches have been utilized to design LFAs for detecting the H5N2 Influenza virus[52] by changing the antibodies specific to the H5N2 virus.

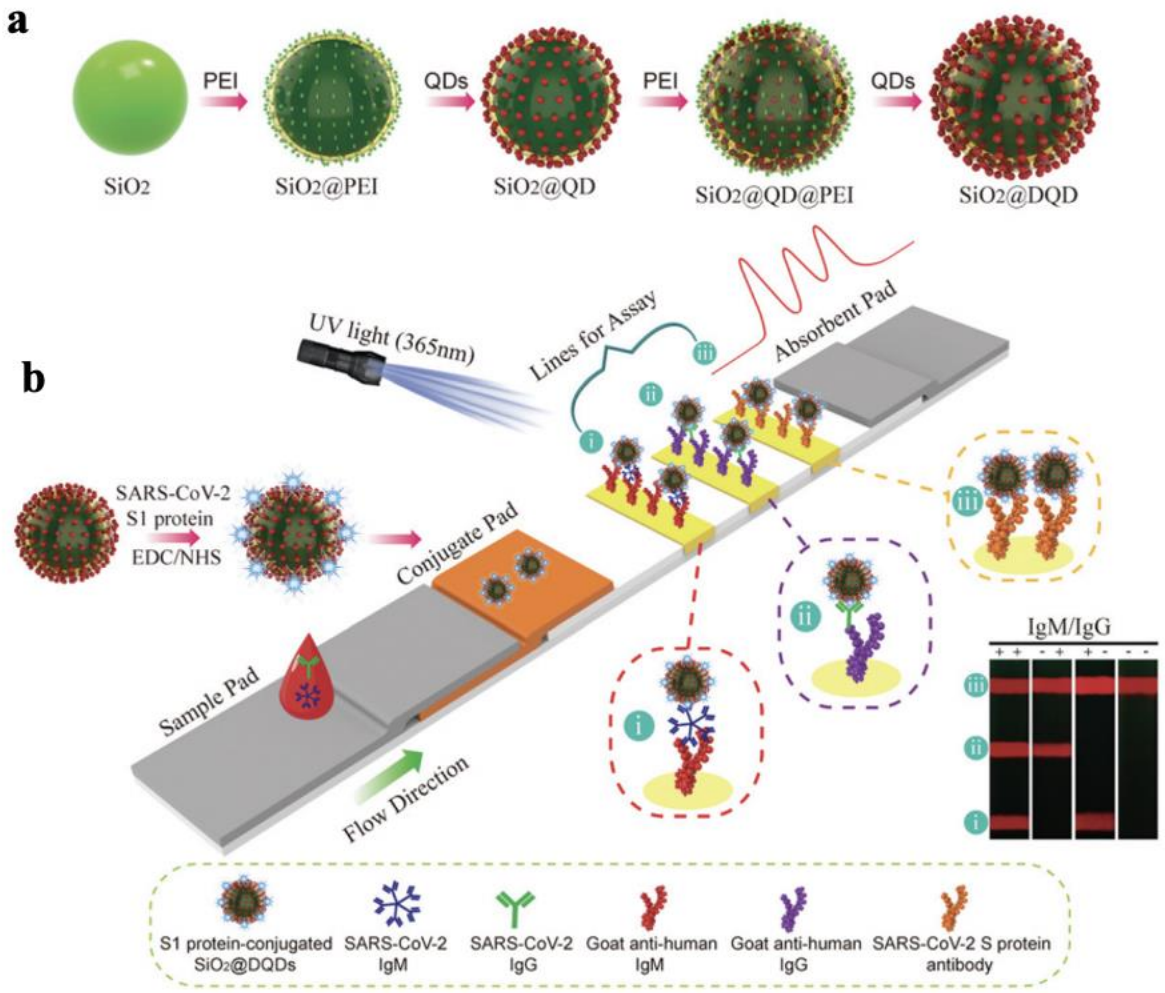

Figure 4. Schematic representation of (a) the synthesis of dual quantum dot (DQD) shell using SiO_2 NP (SiO_2@DQD). (b) Design of LFA strips to detect SARS-CoV-2 antigens using SiO_2@DQD [51].

In another study, Rong et al.[53] developed an integrated fluorescent LFA platform (**Figure 5**) for multiplexed detection of four antibodies (HCV, TP, HIV, and HBsAg). The assay was able to detect very low levels of these antibodies, with the lowest detection limit for HCV, TP, HIV, and HBsAg were 0.14 NCU/mL, 0.62 IU/L, 0.11 NCU/mL, and 0.22 IU/mL, respectively. The test was able to provide results within 20 min.

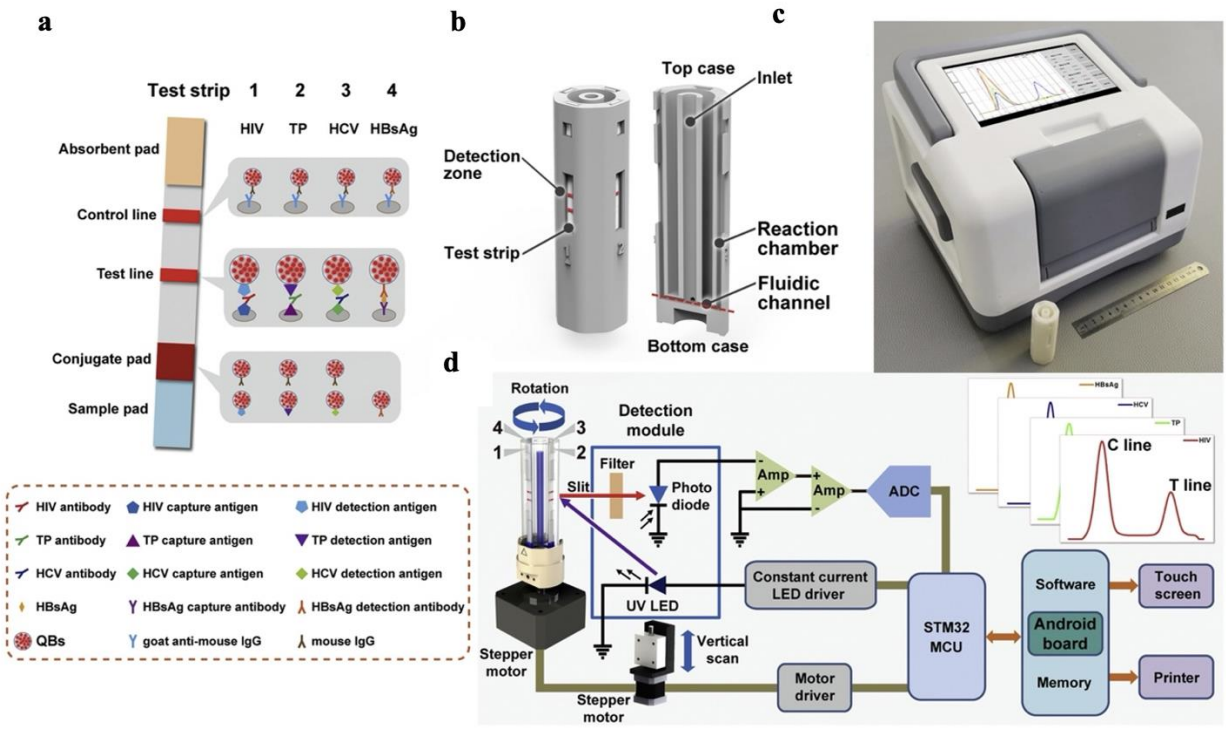

Figure 5. Fluorescence-based LFA. (a) Schematic of the multiplexed LFA device for infectious disease detection. Optical image of the (b) disposable LFA cartridge, (c) LFA reader, and (d) circuit design of the read-out system [53].

3.4 Thermal contrast reader-based LFAs

The light-to-heat conversion of metallic nanoparticles after exposure to a NIR laser is the basis for operating Thermal Contrast Readers [27]. This method uses thermal contrast

amplification (TCA) to improve the sensitivity of the traditional colorimetric LFAs. It utilizes surface plasmon resonance of gold nanoparticles under laser irradiation [16]. Here, the main investigation points are the thermophysical and biological effects of aqueous media heated by laser-activated gold nanoparticles (GNPs) [54].

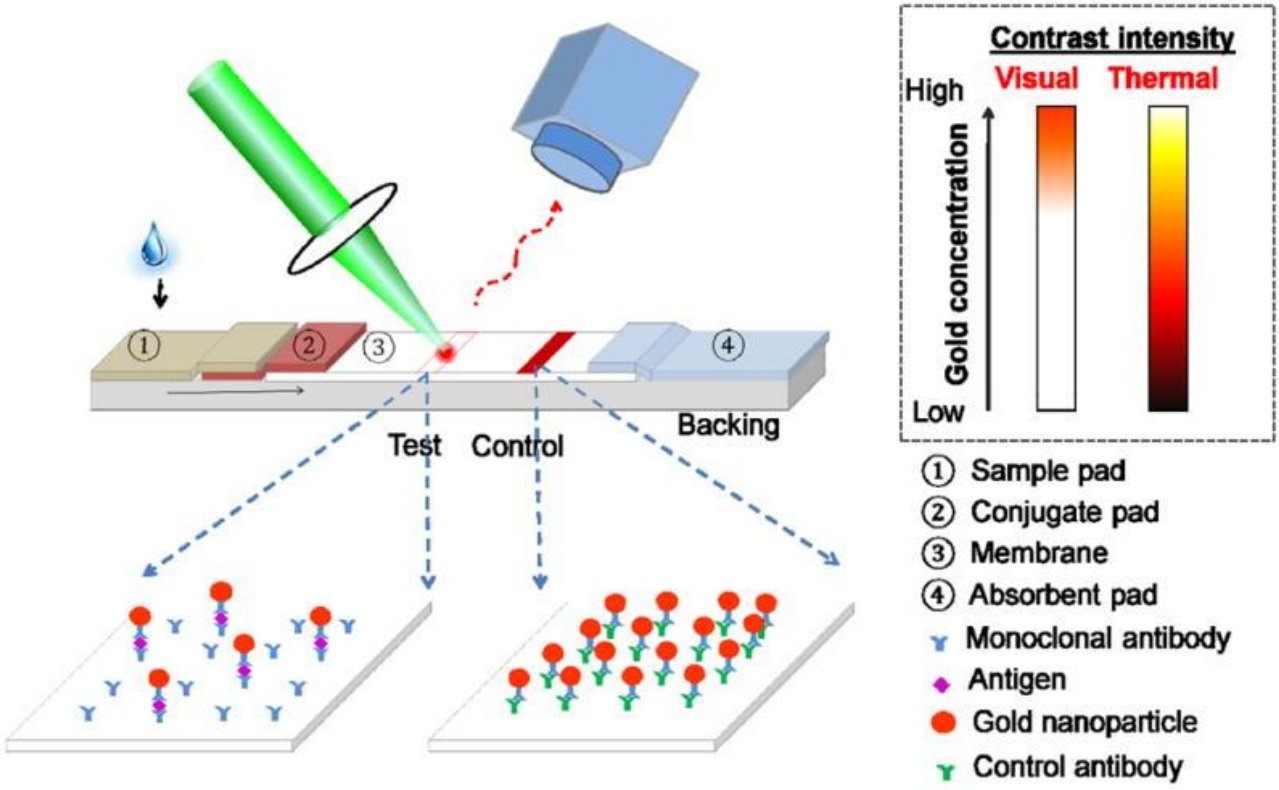

Figure 6. Thermal contrast amplification (TCA) principle and system [55].

The TCA reader is designed to align its laser wavelength precisely with the peak of the GNPs plasmon resonance. This alignment results in the generation of heat, which leads to an increase in temperature. The infrared detector within the TCA reader can monitor this temperature increase [56]. This heat production is proportional to the laser intensity, wavelength, GNP geometry, and concentration. As a result, we can accurately identify and measure the GNP signal for a known disease biomarker [56]. A TCA reader system has three main components according to their functions – IR camera, Green Laser (as the plasmon resonance of GNP is ~ 525 nm), and Control. LabVIEW is generally used for position and time control, and specific holders are designed to house the LFAs [16]. An example of a TCA-based readout is shown in **Figure 6**.

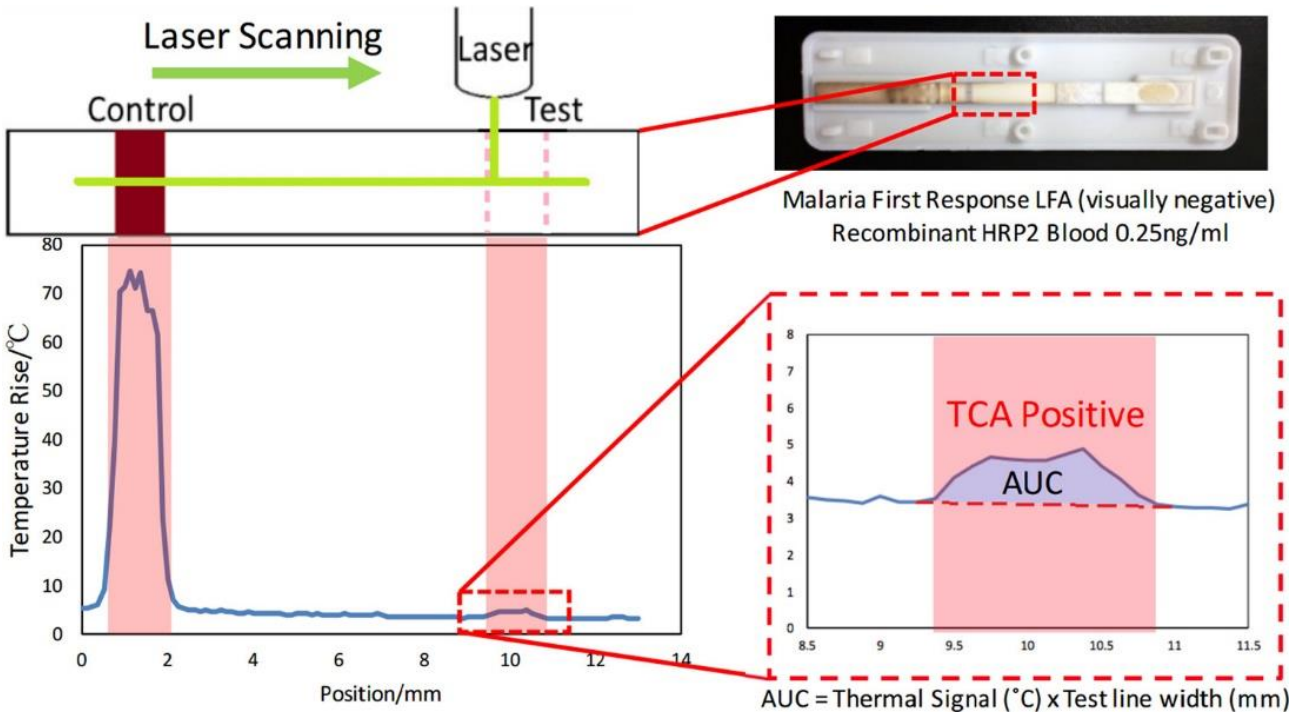

Figure 7. The TCA reader utilizes the area under the curve (AUC) at the test line to measure and identify the temperature rise in a lateral flow assay (LFA) [16].

Wang et al. [16] (**Figure 7**) demonstrated the utilization of the area under the curve (AUC) at the test line location in the LFA as a means to obtain a thermal signal. In this proof of concept, the TCA reader successfully validated a visually negative result of a malaria First Response lateral flow assay (LFA) as a true positive. This LFA's thermal signal score was 1.144 °C as opposed to the negative control's average of 0.179 °C (SD = 0.053).

3.5 Magnetic nanoparticles-based LFAs

Magnetic LFAs combine the speed and ease of LFAs using magnetic nanoparticles (MNPs) as markers. MNPs are particles with a surface that has been functionalized to specifically attach to target analytes. Additionally, these particles possess a magnetic core typically composed of iron oxide. Magnetic readout methods include giant magnetoresistance (GMR)[57], tunneling magnetoresistance (TMR), and magnetic particle quantification (MPQ)[58]. Due to its capacity to recognize the magnetic signal produced by the MNPs, magnetic sensors are frequently utilized in magnetic LFAs. These sensors can quantify or qualitatively describe changes in magnetic fields or magnetic characteristics brought on by the presence of MNPs. In their study, Nguyen et al. [59]

conducted a comprehensive review encompassing these sensors (**Figure 8**). The GMR effect, observed in multilayers with alternating ferromagnetic and nonmagnetic conductive layers, leads to significant changes in electrical resistance based on the orientation of neighboring ferromagnetic layers. When applied, magnetic fields interact with MNPs, resulting in modifications to the measured electric resistance. This enables the quantification of MNPs in GMR-based detection for lateral flow tests. GMR sensors have been demonstrated to detect human chorionic gonadotropin (hCG), interferon gamma (IFN-γ), and cardiac troponin I (cTnI) with greater sensitivity compared to traditional colorimetric detectors [60, 61]. However, GMR-based LFA has limitations, including the need for precise spacing between the sensing element and MNPs, limited reusability, susceptibility to damage or contamination from toxic chemicals, and low dose-response sensitivity that requires improvement [59]. To overcome these limitations, TMR sensors were developed. TMR sensors are more sensitive than GMR sensors and function based on the spin-dependent tunneling phenomenon[62]. The MNPs-embedded assay chip is positioned between magnets in TMR-based detection for lateral flow assays, and the magnetic field passing through the chip causes a change in resistance of the nearby TMR sensor. The biomarkers bound to the MNPs can be quantitatively measured by monitoring the subsequent resistance changes. Compared to GMR sensors, TMR sensors offer a better magnetoresistance ratio and less field noise. Lei et al. [63]created a prototype TMR sensor to detect hCG at a 25 mIU/mL concentration.

MPQ sensors, a type of magnetic-LFAs, assess the nonlinear magnetization response of particles when subjected to a magnetic field to quantify them. The concentration of particles can be determined by measuring the induction response they generate in an alternating magnetic field. By combining two AC frequencies (f1 and f2), sensitive and reliable measurements can be achieved at specific combinatorial frequencies [64]. This method enables sensitive measurement of analytes based on the relative changes in magnetic susceptibility. For the identification of prostate-specific antigen (PSA), Orlov et al.[65] created an immuno-magnetic MPQ-based LFA detector. The results showed a sensitive and linear response. With a linear response spanning a 7-order shift in magnetic particle mass, the LOD observed for 200 nm magnetic beads was 60 zmol. The MPQ sensor can monitor membrane-dwelling particles, which is challenging with traditional optical detection techniques. The MPQ detector has also been used in multiplexed detection for the detection of cardiac biomarkers and the identification of several botulinum neurotoxins.

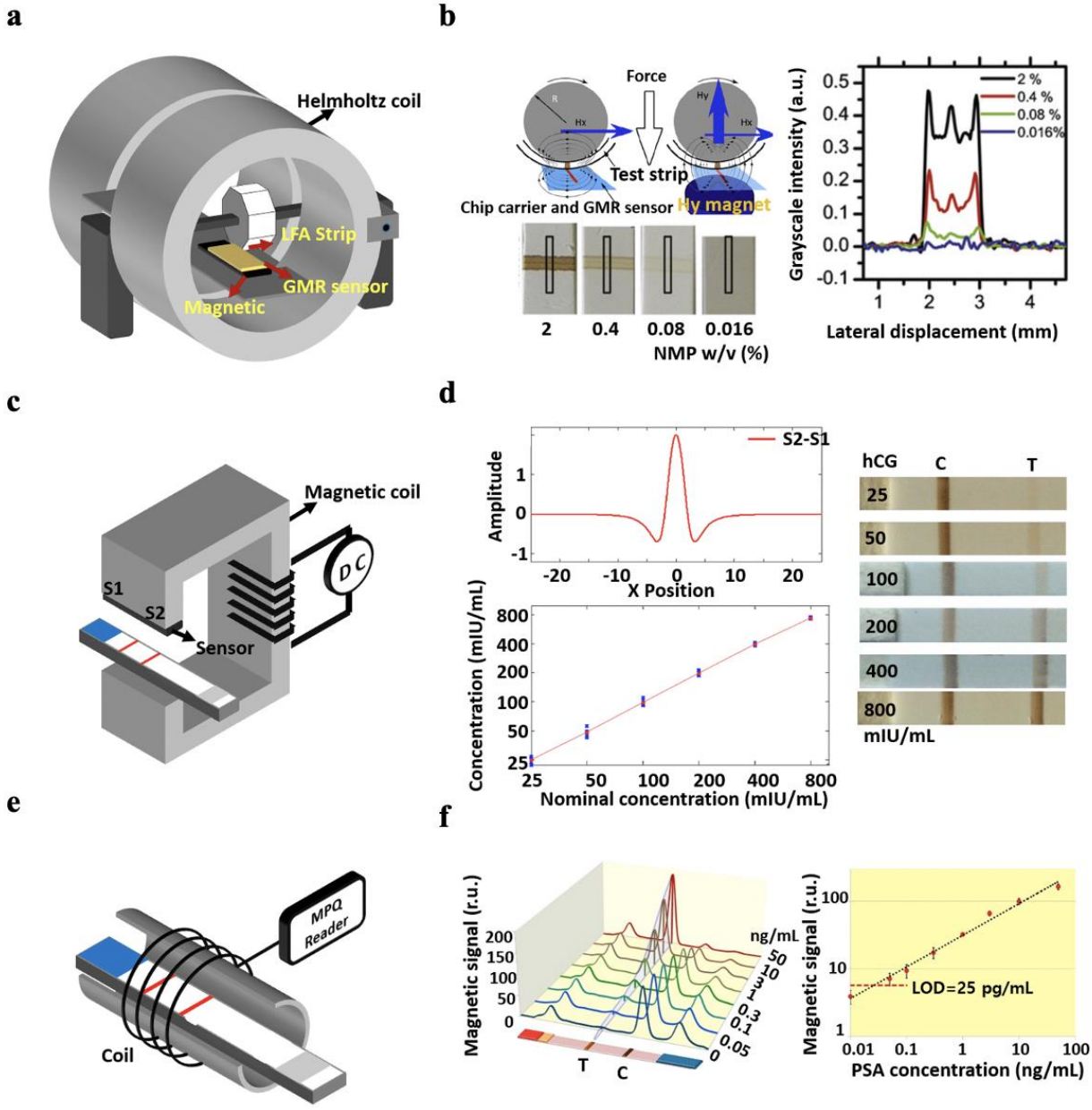

Figure 8. Enhancing the sensitivity of LFAs using a magnetic field. Schematic representation of LFA using (a) giant magnetoresistance (GMR) [57], (b) an example of GMR-based LFA device and results. (c) Schematic representation of LFA using tunneling magnetoresistance (TMR), (d) representative results from a TMR device. (e) Schematic representation of magnetic particle quantification (MPQ)-based LFA, (f) representative results from an MPQ device [59].

3.6 Smartphone-based LFAs

Smartphone-based lateral flow assay (SLFA) significantly affects many different types of point-of-care medical diagnostics. LFAs for infectious diseases, cardiac and tumor biomarkers,

other hormones, or medication screening may all be readily integrated into this system [66]. Gong et al.[35] developed up-conversion nanoparticle-based lateral flow assays (UCNP-LFAs) (**Figure 9**) to improve the sensitivity. The LFA was integrated into a smartphone to improve its usability.

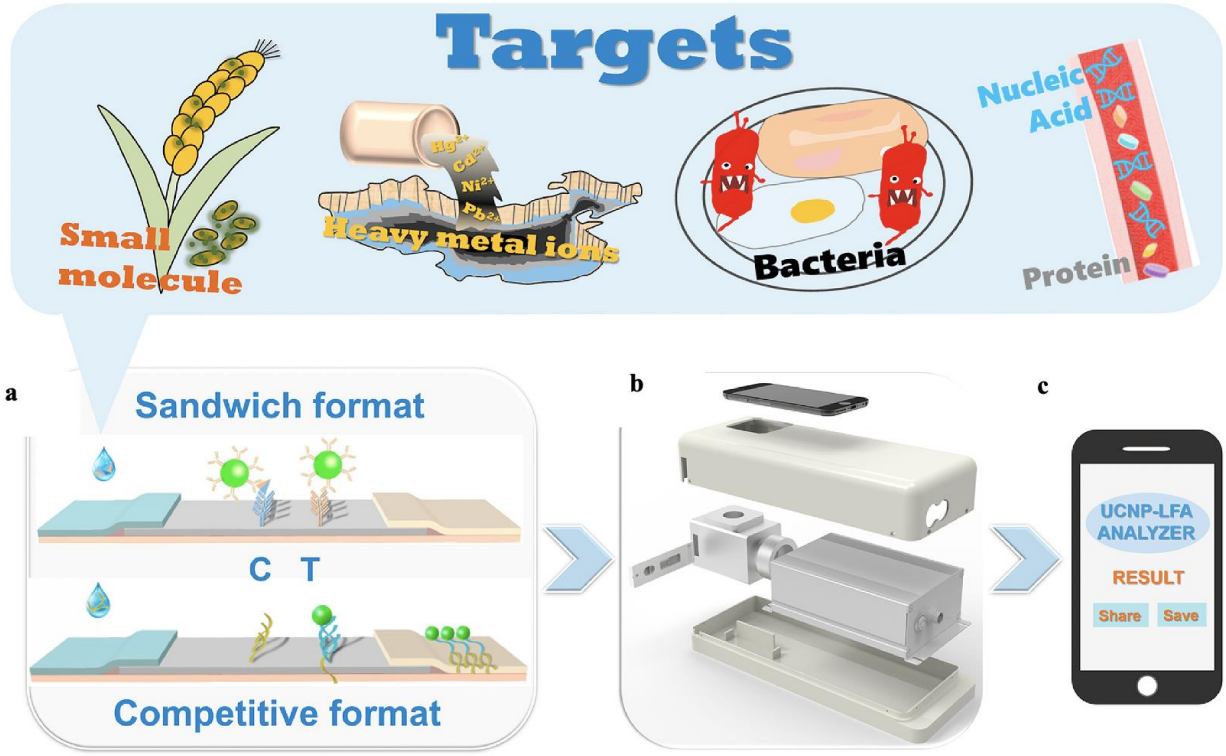

Figure 9. Up-conversion nanoparticle (UCNP)-based LFA. Schematic illustration of UCNP (a) LFA, (b) reader, and (c) smartphone App [35].

Jung et al.[67] proposed a SLFA system that utilizes the smartphone as a colorimetric and quantitative reader. This procedure enhances the classification of the slight variations brought on by various analyte concentrations. While the naked eye cannot reliably verify the existence of a test band, especially at very low analyte concentration, these smartphone readers can detect the test band using image analysis tools. Smartphones can capture a digital image of the experiment's outcome with time and date stamps, which is useful for building databases and subsequent implementation of machine learning (ML)/artificial intelligence (AI) tools. In one example, You et al. [66] utilized a smartphone with a built-in camera to read TSH levels. However, they used the Mie scatter to improve the sensitivity and repeatability of the TSH LFA. They demonstrated a limit of detection (LOD) of 0.3 miu L^{-1} using the Mie scattering technology (**Figure 10**). This made it

possible to identify hyperthyroid conditions, which otherwise need a clinical laboratory. The gadget demonstrated good sensitivity, repeatability, and a testing time of ~ 12 min.

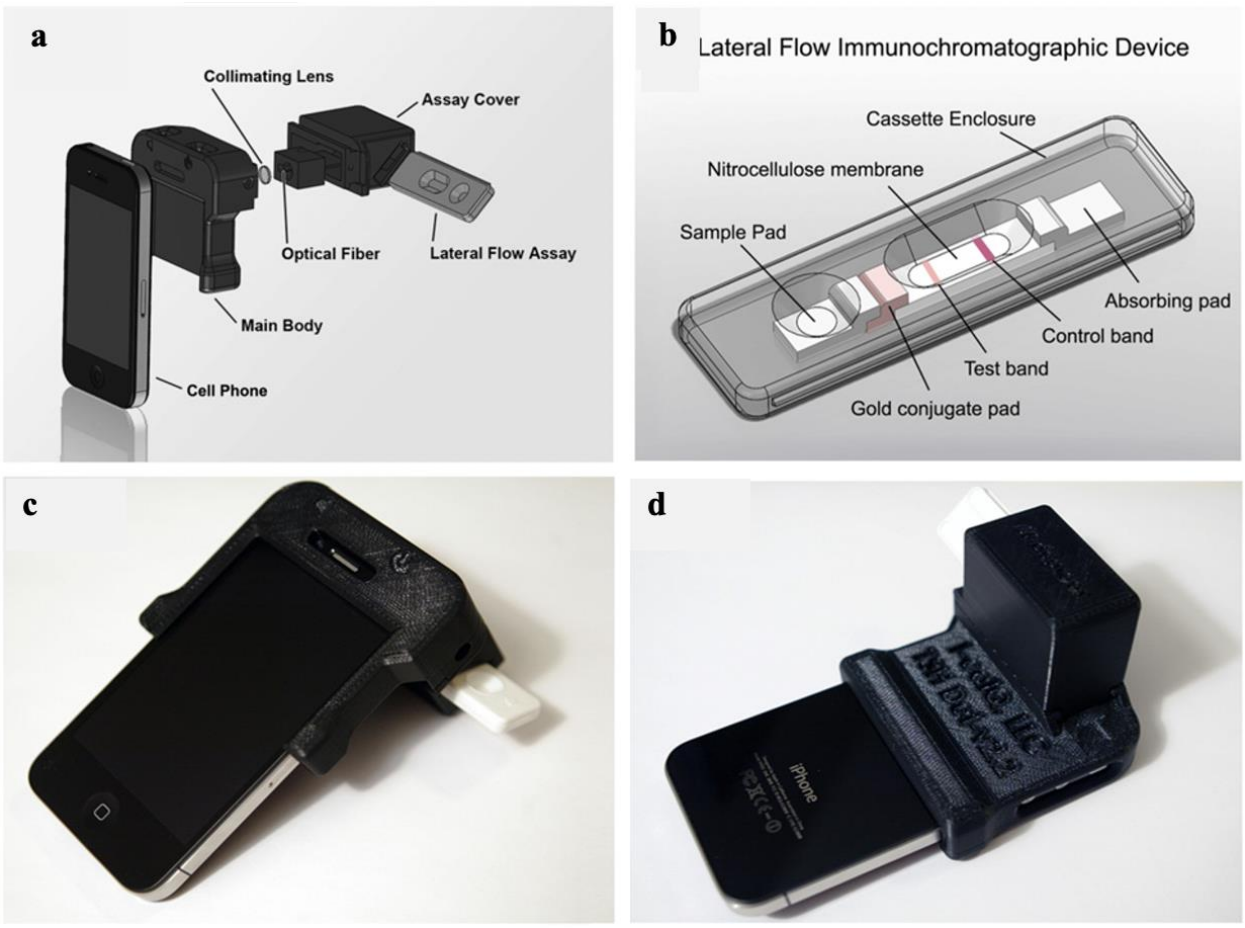

Figure 10. Smartphone coupled LFA detection system. (a) An exploded view shows the smartphone's arrangement, optical system, and LFA sensor. A collimating lens and optical fiber (light pipe) were used to enhance the collection of signal (scattered light from the NPs at the control and test lines). (b) Design of a disposable lateral flow assay test strip for TSH detection. Anti-TSH antibody was immobilized in the test band, anti-IgG in the control band, and gold nanoparticle-conjugated antibodies in the gold conjugate pad. (c, d) Optical images showing the assembled smartphone and TSH detection system [66].

Another study used a smartphone-integrated LFA system for infectious disease detection. Rong et al.[68] developed a platform for the point-of-care detection of nonstructural protein 1 from Zika virus (ZIKV NS1) using fluorescent LFIAs coupled to a smartphone. The study demonstrated the ability of a 3D-printed attachment to integrate external optical and electrical components with

a smartphone, resulting in downsizing and cost savings. This method allowed for quantitative detection of ZIKV NS1 at the point of care in less than 20 min., with limits of detection (LODs) of 0.15 ng mL^{-1} for serum and 0.045 ng mL^{-1} for buffer. **Figure 11** presents the smartphone-based imaging system used in the ZIKV NS1 study. To generate the fluorescence signal, QD microspheres were used. The excitation wavelength of λ = 365 nm was achieved using a high-power UV LED (5 W). To reduce direct reflection, the incident angle of UV light was set to 60°. Improved signal-to-noise ratio (SNR) was achieved using a combination of a short-pass filter (425 nm) and a bandpass filter (624/40 nm).

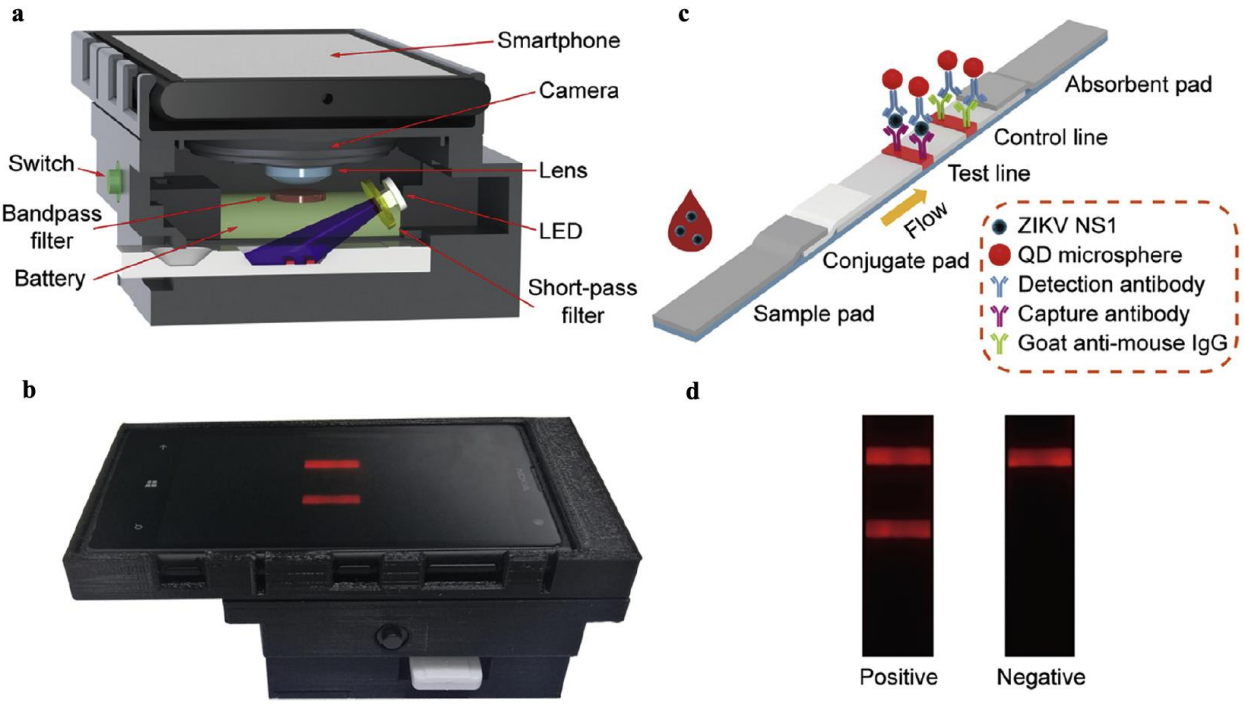

Figure 11. Overview of the design and use of the smartphone-integrated fluorescent LFA platform. (a) A 3D schematic of the internal organization of the smartphone-based imaging equipment. (b) A picture of the luminous LFIA reader in use. (c) Design of the fluorescent LFA for ZIKV NS1 detection. (d) Photographs of the test strips with (positive) and without (negative) ZIKV NS1 [68].

Ross et al.[69] demonstrated a method using Surface Plasmon Resonance (SPR) for selecting crude anti-hazelnut antibodies for use in a quick ligand binding assay based on comparative association rates, cross-reactivity, and sandwich combination capacities. When using

smartphone apps, these LFAs produce semi-quantitative data and a qualitative outcome when read visually. **Figure 12** shows an example of smartphone-based SPR biosensors [69].

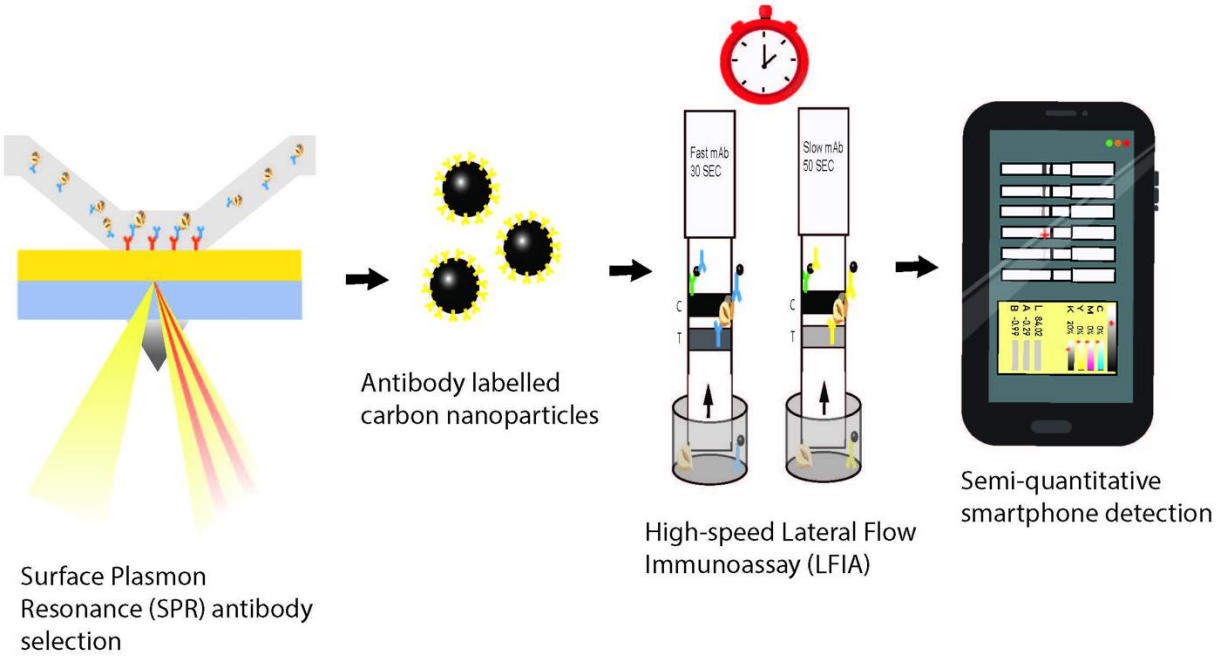

Figure 12. Schematic overview of smartphone-based surface plasmon resonance biosensor [69].

Exploiting smartphones' capacity for SERS detection is vital since it might significantly broaden the applications of LFAs test strips based on SERS [70]. Mu et al.[71] recently developed a smartphone Raman system, in which the spectrometer is integrated into the back of a smartphone, as shown in **Figure 13**, through a slit coupling and rational optical path design. The ease of data sharing is another critical advantage of the smartphone. The collected Raman data can be sent to a computer or cloud over wireless networks for further analysis and processing. The chemical could then be identified using a matching algorithm and the Raman spectrum database. Although the system cost was ~$10,000, it was still significantly lower than a standard laboratory Raman system (which can cost > $50,000) [70, 71].

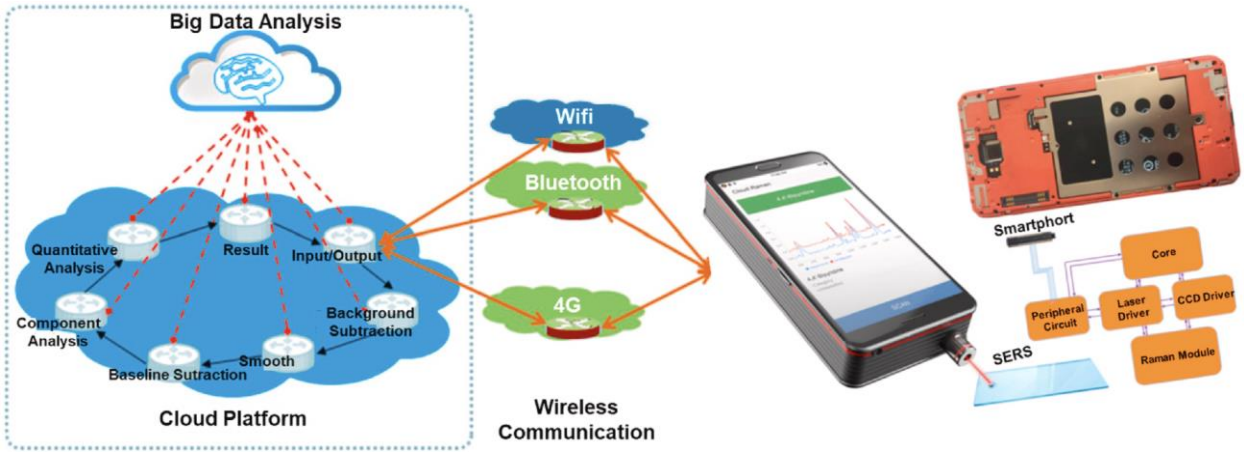

Figure 13. Raman substance detection method for smartphones based on cloud network architecture [70].

4. Incorporating New Technologies and Reagents in LFAs Design

LFAs require several micrometer pores sizes in the paper, limiting the biomolecule capturing capability and assay sensitivity. Most of the LFAs can only detect one biomarker at a time. Due to the complexity of human disease, overlapping symptoms, and co-infection states, there is rising demand for multiplexed systems that can detect many biomarkers simultaneously. Multiplexing may only be possible with a few biomarkers because of inherent restrictions in designing conventional membrane-based LFAs. The multiplexing capability of microarray-based LFAs necessitates precise flow positioning between upstream and downstream spots[72, 73]. There are several strategies that researchers are exploring to increase the sensitivity and specificity of LFAs; some of the most common are discussed in this review. One of the ways to improve the specificity is by multiplexing, which allows for concurrent recognition of multiple analytes in a single sample, as shown in **Figure 14**. Multiplexing on a single stirp has been studied for detecting the Dengue virus NS1 protein, Dengue/chikungunya virus (IgG/IgM)[74], Antibodies against HIV-1 and -2, Mycobacterium[75], Ebola virus, Zaire strain glycoprotein GP[76], Anti-HCV IgG, Anti-HIV IgG, Anti-HAV IgG and IgM[77]. Multiplexing with multiple strips has been studied for E. coli O157:H7, S. paratyphoid A, V. cholera O139, S. paratyphoid C, S. paratyphoid B, S. typhi, S. enteritidis, S. chlorosis, V. cholera O1, and V. parahaemolyticus[78], Influenza A and influenza B virus antigen[79], Pseudomonas aeruginosa, and Staphylococcus aureus[79]. Integration of lateral flow and microarray technologies studied for identifying the malaria antigen plasmodium falciparum histidine-rich protein 2[80].

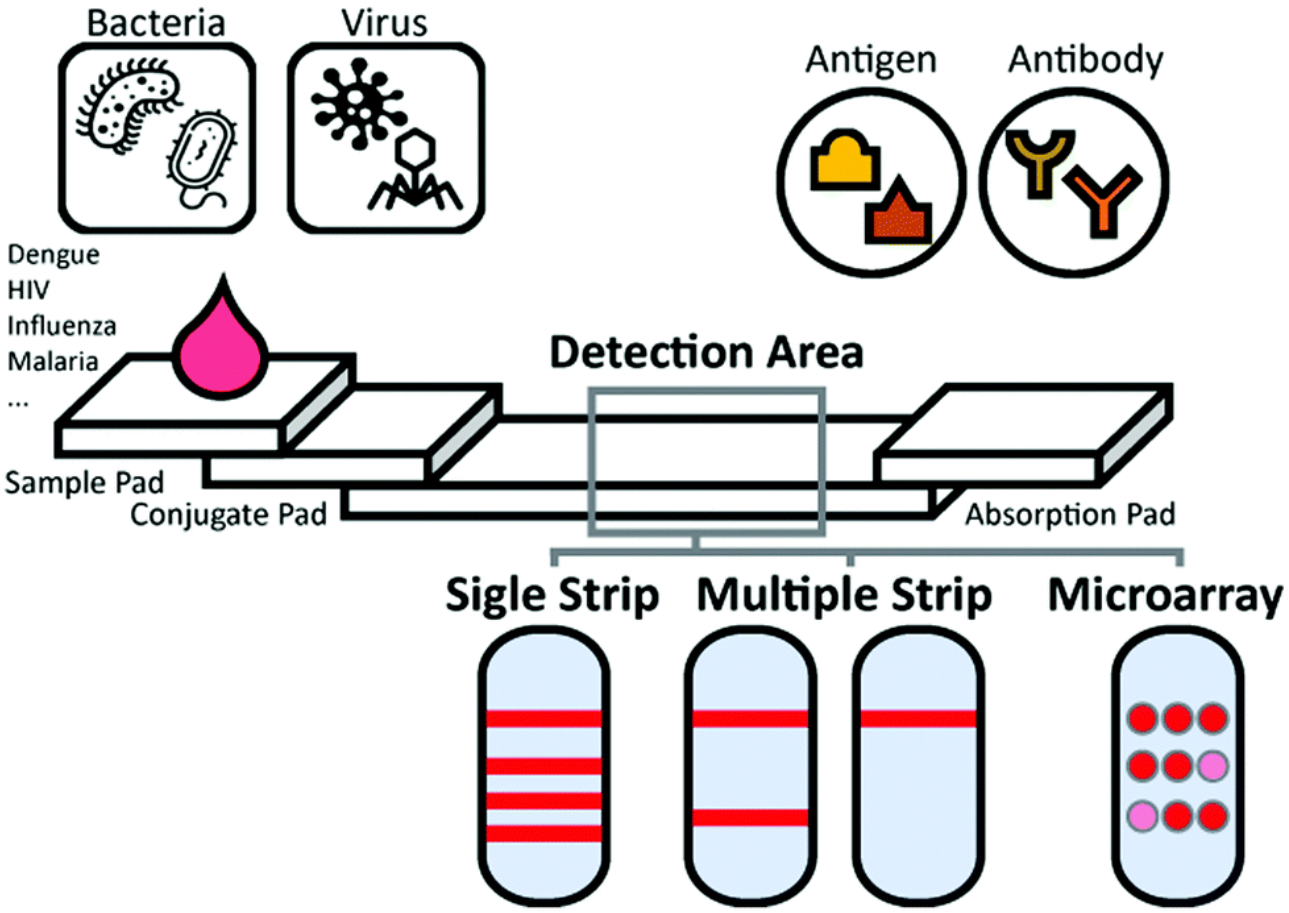

Figure 14. Diagram of a multiplexed lateral flow immunoassay to detect infectious disease biomarkers [81].

Optimally designed nanoparticles (size, shape, materials) can also increase the assay's sensitivity by amplifying the signal generated by binding the analyte to the test line (**Figure 15**). The most frequently used labeling agents in LFAs are antibodies combined with nanoparticles like gold nanoparticles, magnetic nanoparticles[82], carbon-based nanoparticles[83], silver nanoparticles[84], phosphor, fluorescent dyes, quantum dots[85], and up-converting[86] nanomaterials. Gold nanoparticles are the most popular and widely employed for biosensing due to the visible (red) color they create through localized surface plasmon resonance[87, 88]. Aptamers have emerged as versatile alternatives to antibodies in bioanalysis applications due to their structural diversity, adaptability, and non-immunogenic nature. Aptamers offer flexibility in various assay formats and can be utilized for the detection of non-immunogenic and toxic targets. For instance, Shim et al.,[89] created aptamer-based lateral flow assays (LFAs) to detect aflatoxin

B1. Cyanine 5-based fluorescence dye was attached to the aptamer to detect aflatoxin B1. By optimizing the assay, they achieved a limit of detection (LOD) of 0.1 ng/mL. Fu et al., [48] developed a highly responsive lateral flow immunoassay method for specific detection of thrombin in clinical analysis. This method utilized gold nanoparticles (GNPs) and a pair of aptamer probes to enhance sensitivity. However, due to the higher cost associated with GNPs, researchers have explored using common dyes like blue dye as alternative labels for protein detection on LFAs [90], [91]. Mao et al. (reference [92]) proposed latex beads instead of GNPs and a blue dye instead of an expensive fluorescent dye to construct the DNA-based LFAs. The LFA was demonstrated for human plasma and achieved a detection limit of 3.75 fmol/L. In another study, Lee et al. (reference [74]) employed two-color latex beads to detect acute febrile illnesses (AFIs).

mRNA (messenger RNA), an alternative reagent, has gained significant attention recently, particularly in molecular diagnostics[93]. Rapid and precise detection of RNA targets, such as viral RNA or certain gene expressions, is a benefit of mRNA-based LFAs[94]. Infectious disease diagnosis or gene expression[95] profiling is made possible by capturing and detecting mRNA targets on the test line using complementary probes or primers. Similarly, DNA-based reagents LFAs are used to find specific DNA sequences or genetic variants. DNA targets can be identified and captured on the test line using DNA probes or primers. Applications made possible by this include genetic testing[96], pathogen detection[97], and the discovery of specific genetic variants linked to diseases[98]. LFAs may also contain synthetic polymers to enhance the assay performance [99]. These polymers can improve the sample's flow dynamics[100], the test line's ability to bind more substances[101], or the stability of the assay's constituent parts. The sensitivity and dependability of LFAs can be improved by modifying the composition and characteristics of the synthetic polymers[99]. Other reagent enzymes are essential components in many LFAs as they amplify signal [102] and allow for visual or quantitative detection of the target analyte. Substrate molecules can be transformed by enzymes into a visible signal, such as a color shift or the production of fluorescence. Horseradish peroxidase and alkaline phosphatase are usual enzymes in LFAs[103]. These enzymes can catalyze particular reactions that result in a measurable or visual signal, allowing for the sensitive detection of the target analyte. The choice of reagents in LFAs depends on the assay's specific requirements, including the target analyte's nature, the desired sensitivity, and the detection method employed. Researchers continuously explore and

develop novel reagents and strategies to improve the performance and versatility of LFAs for a wide range of applications in diagnostics and biosensing[104-106].

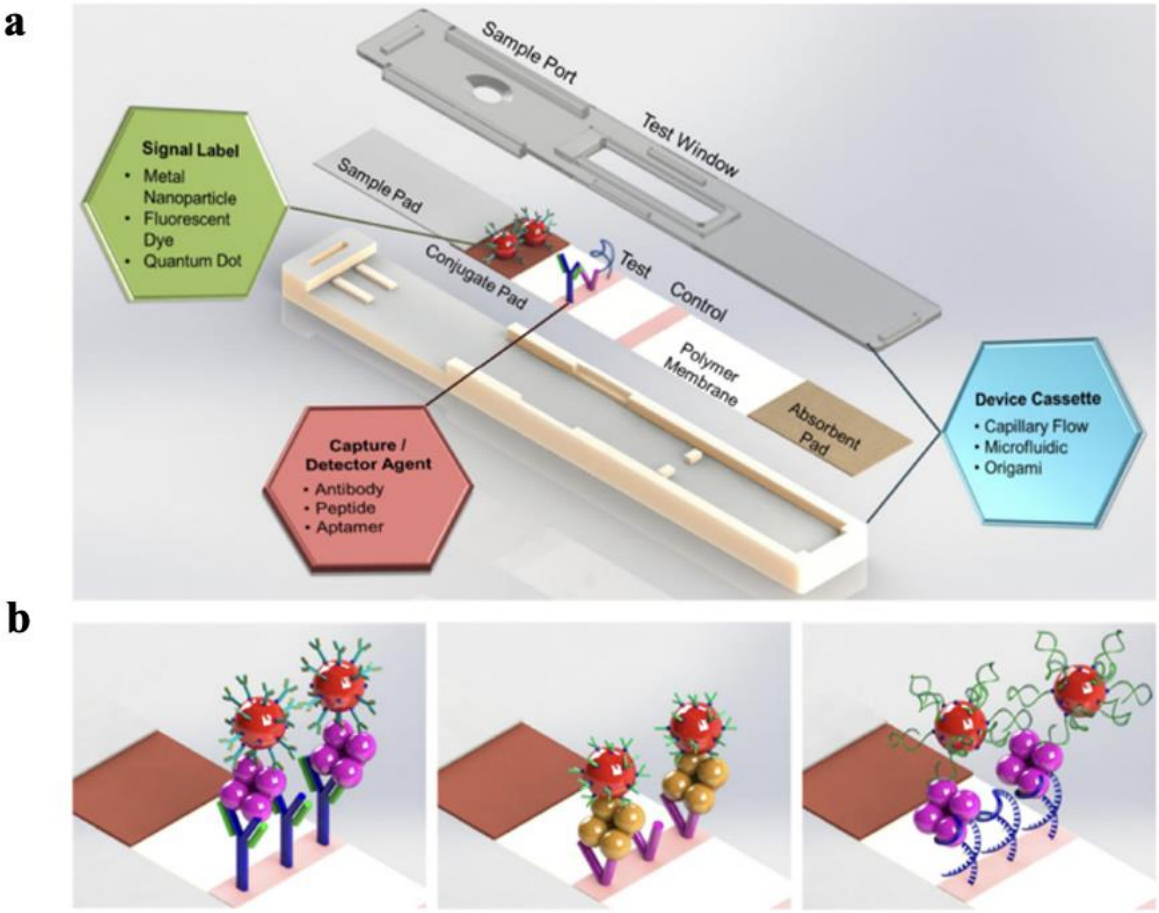

Figure 15. (a) The schematic illustrates the overall design of an LFA test kit and its components (represented by colored boxes), which can be enhanced and improved. (b) In the test line, a sandwich complex is formed when the target molecule binds to the antibodies (left), peptides (middle), or aptamers (right) used as capture and detector agents [107].

Researchers are also exploring the use of signal amplification methods, like enzyme-linked immunosorbent assay (ELISA) or PCR, to increase the sensitivity of LFAs [108]. Santiago-Felipe et al. proposed recombinase polymerase amplification (RPA) with ELISA for screening common food safety threats, such as allergens, genetically modified organisms, pathogenic bacteria, and fungi. It was found that the technique had a satisfactory level of sensitivity and reproducibility. This technique eliminates the need for thermocycling and is cost-effective, making it an ideal method for resource-limited settings[109].

Some research is being pursued on label-free detection methods. These are typically created on the assessment of differences in the physical or chemical properties of the test line, such as changes in refractive index, surface plasmon resonance or impedance, which can increase the sensitivity of the LFAs. Li et al.[110] developed a new method that combines the lateral flow test strip technique with fluorescence immunoassay to detect avian influenza virus (AIV). This method does not require labels but uses a multi-functional nanocomposite to generate signals. This nanocomposite featured a unique combination of magnetic-adhesion-color-nanozyme properties, and it was able to detect the highly pathogenic Escherichia coli O157:H7 with limits of detection of 102 and 10 CFU mL^{-1} for colorimetric and catalytic quantitative analyses, respectively[111]. Tasbasi et al.[112] demonstrated LFA for whole-cell applications (**Figure 16**) to detect Listeria monocytogenes. Finally, the integration of these technologies has allowed for more sophisticated data analysis, providing better insights into the causes of diseases and conditions.

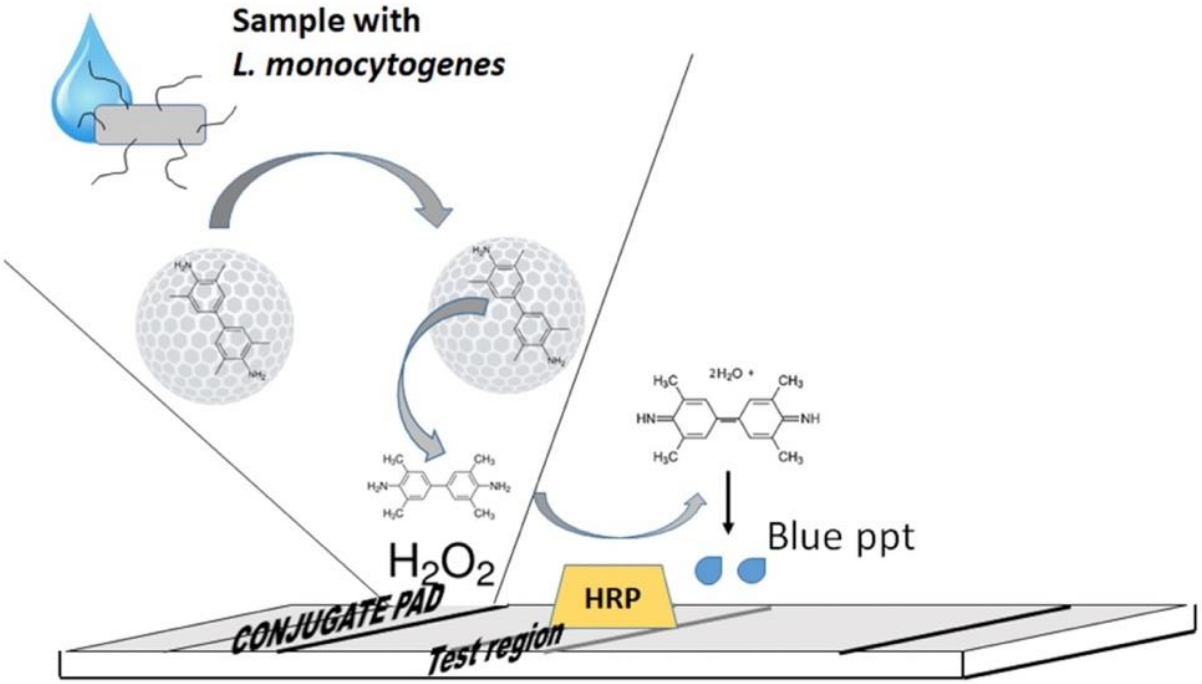

Figure 16. The assay principle is depicted schematically. L. monocytogenes cells move to the conjugation pad region to bind to aptamer gates. Peroxidase immobilized at the test region uses TMB from a nanopore to produce blue precipitates [112].

4.1 Sensitivity enhancement strategy

Lateral flow assay devices are popular for medical diagnostics, but their limited sensitivity can cause false-negative results. To improve their performance, sensitivity enhancement strategies have been developed, including modifications to the device design and optimization of assay parameters. The goal is to increase the detection limit and accurately detect low levels of analytes. Two alternative formats of enzyme-linked immunosorbent assays that can be incorporated into LFAs are sandwich LFAs and competitive LFAs. Even though each style has benefits and drawbacks, sandwich LFAs are more frequently employed than competitive LFAs[113]. In sandwich-type LFAs, the target analyte is caught in a sandwich-like fashion between two distinct antibodies, often called the capture and detection antibodies. The target analyte in the sample is bound to the capture antibody. The detection (or recognition) antibody then attaches with a separate epitope on the target analyte, creating a "sandwich" complex[8]. The detection antibody is tagged with an enzyme or another detectable marker (e.g., fluorescence dye). Thanks to the enzyme-linked detection antibody (or the fluorescent signals), the target analyte can be quantified. By utilizing two distinct antibodies to collect and detect the target analyte, the sandwich format enables the detection of low quantities of the analyte[114]. Sandwich LFAs are appropriate for qualitative and quantitative assessments since they can cover a broad range of analyte concentrations. Different capture and detection antibody combinations can simultaneously detect several target analytes[6]. Since the detection antibody does not directly compete with the analyte in the sample, potential interfering substances in the sample are less likely to affect the assay performance. A labeled analyte (the tracer) is created in a competitive LFA. The labeled analyte competes with the analyte present in the sample to bind to the immobilized capture antibody. The idea is that less tagged analyte will attach to the capture antibody if more analyte is present in the sample[8]. The analyte amount in the sample has an inverse relationship with the signal that is detected. Since the labeled analyte competes with the analyte in sample for binding to the capture antibody, competitive LFAs often has lesser sensitivity compared to sandwich LFAs[115].

Competitive LFAs are better suited for qualitative or semi-quantitative measures rather than accurate quantification because they have a more constrained dynamic range[116]. The competitive format necessitates optimizing several variables, such as the labeled analyte concentration, incubation periods, and the capture antibody to labeled analyte ratio[117]. The development and interpretation of assays may be more difficult due to this intricacy. Considering

the higher sensitivity, more comprehensive dynamic range, and more straightforward assay design, the sandwich LFAs design is generally preferred over the competitive LFAs design. However, it's worth noting that the preference of LFAs format varies on the specific requirements of LFAs, including the target analyte, the desired sensitivity, and the available resources. Table 4 shows different approaches to enhance sensitivity and their LOD, and applications.

Table 4. Sensitivity enhancement strategy

Sensitivity enhancement strategy	**LOD without using the strategy**	**LOD with using the strategy**	**Application**	**Reference**
Flow rate decrease	5nM	0.5nM	synthetic Zika virus	[118]
	100 ng/ml	0.01 ng/ml	Mouse IgG	[119]
Preconcentration	25 fg/ml	100 pg/ml	Valosin containing Protein	[120]
	10^7 CFU/ml	10^6 CFU/ml	*E. coli*	[121]
Nanoparticle based signal enhancement	8.4×10^{-2} pg/µl	2.2×10^{-2} pg/µl	*E. coli*	[122]
	2000 pg/ml	20 pg/µl	PSA	[123]
Alternative signal transduction (SERS/ Fluorescence/ Photoacoustic/ Thermal)	~ 10^{-14}	8.2×10^{-19} M	Biotin	[124]
	10000 pfu/ml	50 pfu/ml	H1N1	[125]
	1.1 ng/ml	0.01 ng/ml	Cryptococcal antigen (CrAg) from Cryptococcus	[17]

4.2 Comparison of multiplexing strategy

Multiplexing is a valuable tool in diagnostic tests, enabling the simultaneous analysis of multiple analytes in a single sample. Multiplexing finds utility in various fields such as food, environment, and safety applications. Additionally, it helps conserve sample volume, time, and cost, which is particularly advantageous as diagnostic samples are often limited in volume [27]. However, the widespread use of multiplexing in LFAs is hampered by several issues [126]. Cross-reactivity, for instance, is a notable challenge as it can lead to non-specific binding, increased background noise, and the generation of false positive results. This phenomenon has been noted in different infectious diseases, including flavivirus infections (such as dengue and Zika virus) as well as helminthic infections (like strongyloidiasis and filariasis). Interestingly, these infections often exhibit overlapping symptoms, further complicating the accurate detection and differentiation of specific biomarkers in the multiplexed LFAs [127, 128]. The quantity and variety of biomarkers that can be included in a single LFAs are constrained by this restriction. Physical limitations arise from the inadequacy of multiplexing multiple test lines on a single LFA strip due to conjugate quantity, fluid flow variations, and bypassing multiple lines [129]. Converting existing single-biomarker LFAs to multiplexed LFAs brings operational and quality assurance challenges, including patient and control population selection, calibration techniques, and process validation. The interpretation of multiple test results complicates LFAs, undermining their inherent simplicity. The proprietary nature of single-test LFAs' biomarkers hinders the sharing and teamwork required for developing multiplexed LFAs. Although promising prototypes have been developed in research, the translation of multiplexed LFAs into commercial devices remains limited [130], underscoring the necessity of addressing these issues through more research and development efforts [131].

Recent research has seen the emergence of several intriguing reports of multiplexed LFAs prototypes, demonstrating the possibility for simultaneous detection of several targets. Yen et al., [76] proposed a multiplexed LFA that could simultaneously detect the viruses that cause dengue, yellow fever, and ebola by using multicolored silver nanoparticles (AgNPs) to create different colored test lines. Another cutting-edge design included a disc format with 10 distinct dipsticks [129], each lined with a different biomarker for detecting foodborne pathogens. Up-converting phosphor (UCP) particles were used in this design as the reporter to enable increased sensitivity

and sample interference tolerance. The promise of multiplexed LFAs in the realm of immunology was also demonstrated by a multiplexed LFA that was created using UCP technology to detect several cytokines in leprosy. Lee et al., developed a multiplexed test with different viral antigens for diagnosing AIDS, hepatitis C and A (HCV, HAV), and HCV in one test strip [77]. By enabling the simultaneous detection of several targets, these extraordinary developments in multiplexed LFAs emphasize the potential for increased diagnostic capabilities, leading to more efficient disease management and control. Table 5 shows the comparison of different multiplexing strategies for LFA devices.

Table 5. Comparison of multiplexing strategy

Strategy	Transduction method	Number of targets	Biomarker Detected	Application	Sample type	LOD; enhancement	Ref.
Antibodies on multiple test lines	SiO2@DQD,	2	HIgG, HIgM	SARS and Cov-2	human serum	10^4-fold	[51]
	FAM and ROX fluorophores	13	HPV(16,18,31,33,35,39,45,51,52,56,58,59, and 68	medical diagnostics, food safety testing, and environmental monitoring	human cervical swab	No	[12]
	AuNPs	3	miRNA-21, miRNA-155, miRNA-210, Giardia, Cryptosporidium, Entamoeba, HIV, HCV, and HAV antibodies	cancer, cardiovascular disease, and neurological disorders	human serum and stool	No	[132]
Microarray	AuNPs	4	morphine, amphetamine, methamphetamine, and benzoylecgonine	monitoring of drug	human urine	No	[133]
Multiple strips	UCP nanoparticles	10	S. paratyphi A, E. coli O157:H7, S. typhi,S. paratyphi B, S. paratyphi C, S.	dairy products, marine products, beverages, snacks, and meats	food samples	No	[129]

			enteritidis, V. cholera O1, S. choleraesuis, V. cholera O139, and V. parahemolyticus				
Multiple channels	catalytic signal amplification of AuNPs	3	Clostridioides difficile toxins A and B and glutamate dehydrogenase (GDH)	Clostridioides difficile infection	human stool	8-fold	[134]
Multiple dots	AuNPs	7	DNA alleles (GYPB*03, GYPB*04, FY*01, FY*02, FY02N.01, JK*01, and JK*02) related to four blood group SNPs	blood group detection	human whole blood	No	[135]
Multiple antibodies on a single test line	Ag^{NBA}@Au, Ag^{MB}@Au, and Ag^{R6G}@Au SERS nanotags	3	CK-MB, cTnI, and Myo cardiac biomarkers	myocardial infarction	human serum	100-fold	[136]
	Red, orange, and green AgNPs	3	ZEBOV glycoprotein, YFV NS1 protein and DENV NS1 protein	dengue, yellow fever, and Ebola viruses	human serum	No	[76]

4.3 Application of LFA in various infectious diseases

Lateral flow assays (LFAs) are frequently utilized to diagnose different types of infectious diseases, such as viral, bacterial, and parasitic infections. By detecting a diverse range of biomarkers, such as antibodies, antigens, and nucleic acids, LFAs offer rapid and precise diagnosis of infectious diseases in various clinical settings. Table 6 compares the metrics relevant to LFAs applied to detect contagious diseases.

Table 6. LFA applied to various infectious diseases

Pathogen Type	**Pathogen Name**	**Type of Approach/ Detection Element**	**Analysis Time**	**LOD**	**Sensitivity**	**Type of LFA**	**Recognition element /Reporter (DNA/ RNA/ protein/ aptamer/ small molecule)**	**Reference**
	COVID-19	Nucleic Acid	30 min	1 ag of the N-gene RNA	-	RT-LAMP Colorimetric	RNA	[137]
			36 min	200 copies/mL	-	Colorimetric	FeS2 nanozyme strips	[138]
			60 mins	200 pM	-	Fluorescent	AIEgen@GO	[139]
			90 min	2000 copies/mL	-	catalytic hairpin assembly (CHA) reaction	DNA-Hairpin	[140]
			-	5 ag/μL	-	Electrochemical	Universal DNA-Hairpin (UDH)	[141]
		Antibody	8 min	100 fg/mL	-	SERS	AuNPs	[24]
			10 min	IgG 5 ng/mL	93.33%	Colorimetric	Selenium nanoparticle-modified SARS-CoV-2 nucleoprotein	[142]
				IgM 20 ng/mL				
			10 min	-	IgG 98.72%	Fluorescent	Eu(III) fluorescent microsphere	[143]
					IgM 98.68%			

			10 min	-	IgG 100% IgM 92%	Colorimetric	AuNPs	[144]
			15 min	IgG 0.1 ng/mL IgM 1 ng/mL	-	SERS	Gap enhancement nanotags	[145]
		Antigen	10 min	-	100%	Fluorescent	Europium (III) chelate microparticles	[146]
			10 min	1.6-2.2 ng/mL	-	Fluorescent	UCNPs@mSiO2	[147]
			16 min	0.1 ng/mL	-	Chemiluminescent	CoeFe@hemin-peroxidase nanoenzyme	[148]
			20 min	10 pg/mL	-	Fluorescent	Carbon dot-based silica spheres	[149]
			20 min	3.03 ng/mL	-	Colorimetrci	Carboxylic red latex beads	[150]
		Nucleic Acid	15 min	0.76 pM	-	Fluorescent	QDs	[151]
			15 min	0.24 pg/mL	-	SERS	Surface modified AuNP by MGITC	[48]

Virus	HIV		15 min	0.1 nM	-	Colorimetric	Oligonucleotide-linked AuNPs	[152]
			20 min	0.5-13 log10 copies/mL (50 copies of gag RNA)	-	Colorimetricc	AuNPs	[153]
			< 60 min	0.3 fm	-	CRISPR-cas12a mediated SERS	SiO2@PEI NPs	[154]
		Antibody	20 min	0.11 NCU/mL	-	Fluorescent	QBs	[155]
			30 min	-	100%	Proteinticle probe-based Colorimetric	AuNPs	[156]
			20-40 min		96.6%	Colorimetric	UCNPs	[157]
		Antigen	18 sec	0.0064 ng/mL	-	Amperometric	AuNP-MWCNTs-AEP	[158]
			15 min	-	100%	Colorimetric	AuNPs	[159]
			20 min	8 pg/mL	-	Colorimetric	PtNPs	[160]
			40 min	30 pg/mL	-	MICT	AuNPs	[161]
			40 min	50 pg/mL	90%	Colorimetric	Carbon NPs	[162]
	Synthetic Zika Virus	Nucleic Acid	1.5-15 min	0.5 nM	-	Electro-spin Coating and Colorimetric	Hydrophobic PCL Nanofibers	[118]

			15 min	1 copy/μL	-	RT-LAMP Smartphone	Microfluidic Chips	[163]
		Antibody	-	1.905 μg/mL	-	SERS	Si-AuNPs	[164]
			20 min	10 pg/mL	-	Colorimetric	Silanized Carbon Dots	[165]
		Antigen	20 min	0.045 ng/mL	-	Fluorescent	Quantum dot microspheres	[166]
	Influenza A	Antibody	10 min	0.25 HA units	-	Colorimetric	AuNPs	[167]
			20 min	-	78.57%	Colorimetric	Streptavidin-coated gold colloid	[168]
			30 min	50 pfu/mL	-	SERS	Fe_3O_4@Ag magnetic tags	[169]
			35 min	22 pfu/mL	-	Fluorescent	MQBs	[170]
		Antigen	15 min	50 pfu/mL	-	Fluorescent	QDs	[155]
			30 min	250 ng/mL	-	Fluorescent	Cy5 doped silica nanoparticles	[171]
	Influenza B	Antibody	20 min	-	87.50%	Colorimetric	Streptavidin-coated gold colloid	[168]
			30 min	0.55 μg	-	Fluorescent	Cy5-loaded silica nanoparticles	[172]
	Avian Influenza	Antibody	10 min	0.09 ng/mL	-	Fluorescent	QDs-AuNPs	[108]

	Influenza	Enzyme	15 min	995 TCID50/mL	-	Bio chemiluminescent	luciferin derivatized substrate	[173]
	Dengue	Nucleic Acid	20 min	cut-off value of 1.2 104 pfu/mL	-	Colorimetric	Dextrin-capped AuNPs	[174]
		Antibody	15 min	512 pfu	-	Colorimetric	AuNPs	[175]
			-	150 ng/mL	-	Colorimetric	AgNPs	[155]
		Antigen	10 min	4.9 ng/mL	-	Colorimetric	Gold decorated graphene oxide sheets	[176]
			25 min	0.1 ng/mL (DENV 1)	-	Magneto Enzyme LFA-Colorimetric	Carboxyl-Adembeads	[177]
				0.25 ng/mL (DENV 2,3)				
				1 ng/mL (DENV 4)				
			30 mins	7.67 ng/mL	-	SERS	Gold nanostars	[178]
	Hepatitis A and C	Antigen	30 min	-	100%	Colorimetric	AuNPs	[77]
	Hepatitis B	Nucleic Acid	7 min	7.23 pM	-	Colorimetric	AuNPs	[179]
		Antibody	10 min	60 mIU/mL	99.19%	Colorimetric	Up-converting phosphor	[180]
			20 min	2.5−10.0 IU HBV surface antigen/mL	-	Fluorescent	DNA- Fluoro-Max fluorescent nanoparticles	[181]

		Antigen	10 min	0.05 ng/mL	-	Fluorescent	ZnSe/CdSe/CdS/Cd xZn1-xS/ZnS QDs	[182]
			15 min	75 pg/mL	-	Colorimetric	Carboxyl modified CdSe/ZnS QDs	[183]
Bacteria	*Staphylococcus aureus*	Antibody	12 min (testing) + 10 min (chromatography)	104 cells/mL	-	Colorimetric	vancomycin (Van)-modified SiO2–Au-QD tags	[184]
				100 cells/mL	-	Fluorescent		
	Foodborne bacteria	Antibodies	20 min	10 CFU/0.6 mg	-	TC-UPT-LF	UCP	[129]
	Clostridioides difficile	Antibody	10 min	0.16 ng/mL	-	mPAD	glutamate dehydrogenase	[185]
				0.09 ng/mL			toxin A	
				0.03 ng/mL			toxin B	
	Vibrio Cholerae	Antibody	10 min	5 ng/mL (3σ)	-	Colorimetric	aptamer coated AuNPs	[186]
				10 ng/mL (visual)	-			
			20 min	0.6 ng/mL (3σ)	-			
				1 ng/mL (visual)	-			
	Salmonella Enteritidis	Phage-Antibody	30 mins	7.06 CFU/mL	-	SERS + Colorimetric	DTNB-AuNPs	[187]
			15 min	4.1×10^6 CFU/mL	-	Colorimetric	Phage	[188]

	Salmonella typhimurium	Antibody	10 mins	75 cfu/mL	-	SERS	4-MBA-modified AuNPs	[189]
			15 mins	75 cfu/mL	-	Magnetic	AuNR@Pt	[190]
				50 cfu/mL		Colorimetric		
			35 min	30 cells/mL	-	Ultrasensitive Fluorescent	Mag@QDs-WGA	[191]
			15 min	50 cells/mL	-	Fluorescent	Si@DQD	[192]
			90 min	8.6×10^0 CFU/mL (pure culture)	-	Colorimetric	multifold AuNPs	[193]
				4.1×10^2 CFU/mL (real sample)				
	Cronobacter sakazakii	Antibody	3 hrs	10^3 cfu/mL	-	Colorimetric	AuNPs	[194]
	Mycobacterium tuberculosis	Nucleic Acid	20 min	25 fg	100%	Colorimetric	-	[195]
			75 min	-	60%	Colorimetric	-	[196]
	Streptococcus pneumoniae	Nucleic Acid	2-10 min	25 fg/μL	-	Colorimetric	AUDG	[197]
		Antigen	15 min	-	82.8%	Colorimetric	Antibody	[198]

5. Challenges in Incorporating New Technologies and Reagents in LFAs Design

Incorporating new technologies and reagents into LFA design can be a challenging process. One of the primary challenges is achieving sufficient sensitivity for the target analyte[199]. New technologies and reagents may require more complex detection systems than traditional LFAs, which can be costly and challenging to implement[131]. Additionally, as LFAs become more complex, they become more prone to errors and can be more expensive to manufacture. Integrating new components into existing LFA platforms requires careful optimization and integration, which can be time-consuming and costly if the components are not compatible[113]. Environmental conditions such as humidity, temperature, and light can also impact new components' stability, posing additional challenges[200]. Before any new technology or reagent can be approved for use in a diagnostic assay, it must undergo thorough evaluation for safety and efficacy, which can be lengthy and costly. Despite these challenges, successful implementation of new technologies and reagents in LFAs can offer significant benefits. Collaboration between scientists, engineers, and regulatory experts is crucial to ensuring that the assay is effective, reliable, and safe for use in clinical settings.

5.1 Complexity of operation

The complexity of the operation is another critical challenge that can arise when incorporating new technologies and reagents into LFA design[199]. LFAs may require extensive user training to ensure proper assay performance as they become more complex. This can be difficult in point-of-care settings where trained personnel may not be available. Additionally, new technologies and reagents may require additional assay steps, increasing the complexity and time needed to perform the assay, leading to increased costs and reduced throughput. Interpreting assay results may also become more complex with integrating new technologies and reagents, which can be challenging for non-expert users [201].

In some cases, specialized instrumentation may be necessary for implementing new technologies and reagents in LFAs, further adding to the complexity and cost of the assay. Integrating new technologies and reagents into existing LFA platforms requires careful integration and optimization, which requires significant engineering expertise [202]. Addressing the

complexity of operation requires careful assay design, user-friendly interfaces, and robust quality control measures to ensure reliable and accurate results. Therefore, managing the complexity of the operation is critical for successfully implementing new technologies and reagents in LFA design.

5.2 Potential for false positives

The potential for false positives is a critical challenge that can arise when incorporating new technologies and reagents into LFA design. Some new reagents or technologies may cross-react with other substances in the sample, leading to false positive results [203]. This can be particularly problematic in complex samples like blood or urine. In addition, some new reagents may bind non-specifically to LFA components, leading to false positives that can be difficult to detect and correct in the assay design [204]. Contamination of LFA components or the sample can also lead to false positives, which can be challenging to prevent in low-resource settings or areas with poor laboratory infrastructure.

Furthermore, some new technologies or reagents may lack the specificity to distinguish between the target analyte and related compounds, leading to false positive results [205]. The sample matrix can also impact the potential for false positives, as high levels of interfering substances can increase the likelihood of false positives[206]. Addressing the potential for false positives requires careful assay design and validation, including appropriate controls and optimization of assay conditions. Thorough characterization of the target analyte and potential interferents is also necessary to minimize the potential for false positives. Thus, addressing the challenge of potential false positives is expected to improve the accuracy and reliability of LFA design.

6. Conclusion

New technologies and reagents can potentially improve the accuracy and sensitivity of lateral flow assays (LFAs). Surface Enhanced Raman spectroscopy (SERS) and Photoacoustic techniques can enhance the sensitivity of LFAs, while Fluorescence Resonance Energy Transfer (FRET) can enhance specificity. Integrating smartphones and thermal readers can provide quantitative measurements and improve the accuracy of LFAs. However, incorporating these

technologies into LFA design can present significant challenges, such as higher cost, complexity of operation, and potential for false positives. Careful assay design and validation, appropriate controls, and optimization of assay conditions are necessary to minimize the potential for false positives [207]. LFA development is increasingly driven by consumer needs, creating user-friendly technologies that enable easier testing by untrained individuals [208], [209], [210]. Incorporating artificial intelligence and machine learning can help automate the interpretation of test results, making LFAs more accessible to the general population [211]. LFAs technology innovation extends beyond healthcare [212]. Enhancements in accuracy and sensitivity can increase their adoption in other areas, resulting in safer environments, reliable food testing, and effective forensic investigations. Continued innovation in LFAs technology is vital for improving diagnostic tools, expanding their applications, and ultimately enhancing human health and well-being.

Data availability

The data that used in the study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: M.R.G., M.K.D.; ***Data curation:*** M.K.D., M.I.; ***Funding acquisition:*** M.R.G., R.D.; ***Methodology:*** M.R.G., M.K.D., R.D.; ***Project administration:*** M.R.G.; Resources: M.R.G., R.D.; ***Supervision:*** M.R.G., R.D.; ***Writing – original draft:*** M.K.D., M.I.; ***Writing – review & editing:*** M.R.G., M.K.D., M.I., R.D.;

Conflicts of interest

The authors declare no competing financial interests.

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