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 are some ways that LFA accuracy and sensitivity can be improved. To ensure reliable and accurate results, careful assay design and validation, appropriate controls, and optimization of assay conditions are necessary. In the future, LFAs are expected to become even more accessible and efficient, with greater accuracy and sensitivity. 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 health care or clinical diagnosis, animal health, 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 infectious diseases—bacterial pneumonia, syphilis, malaria, and tuberculosis—is thought to be able to avert at least 1.2 million deaths

annually worldwide[2-4]. The most popular point of care (POC) test is the lateral flow test (LFT) which is also known as a lateral flow device (LFD), lateral flow immunochromatographic assay (LFA), or rapid test. This method was first reported in 1956 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 urine[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 produce a signal 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. To check the test's validity and verify the device's functioning, a control line should appear for both positive and negative results. 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.

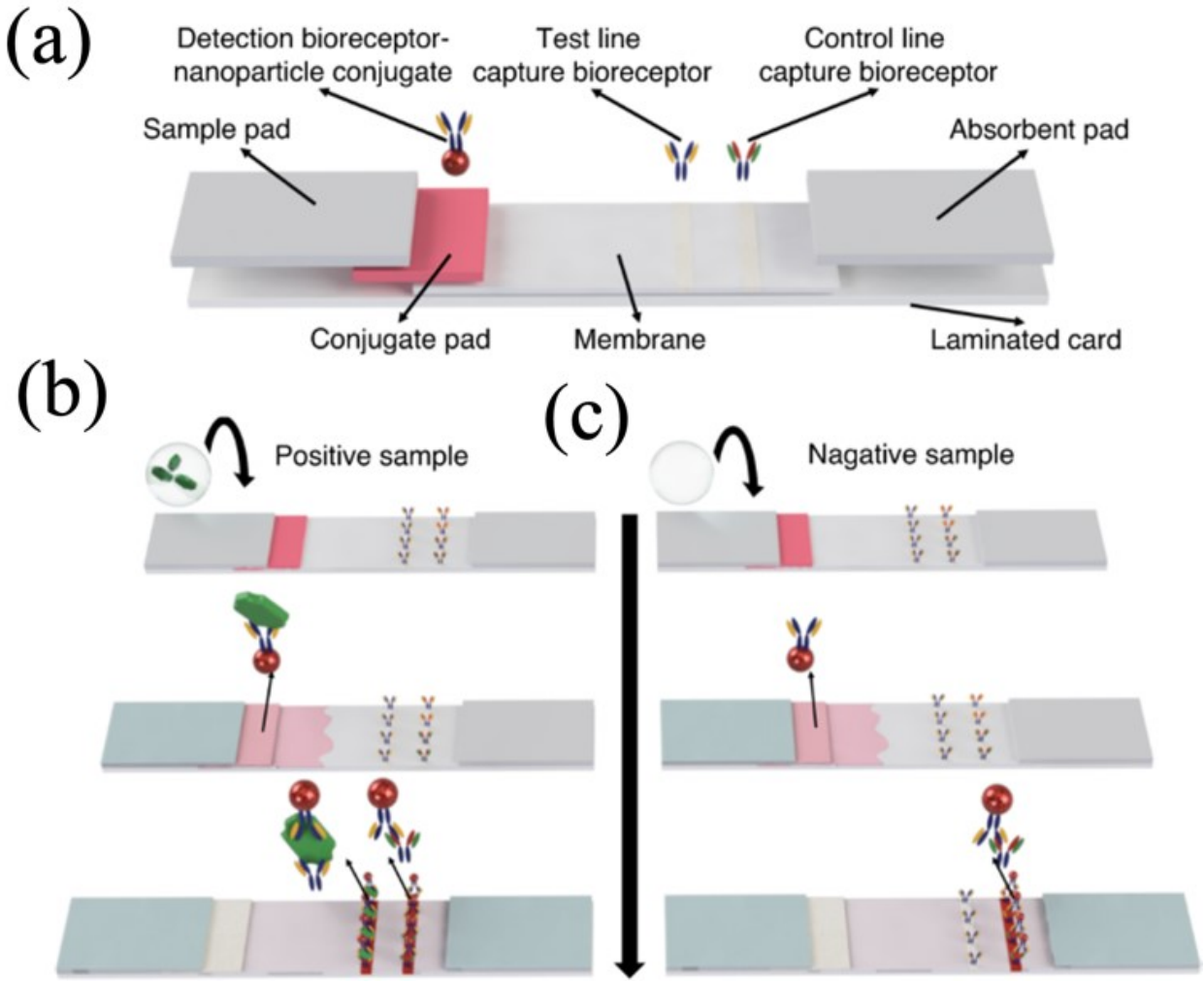

Figure 1. Schematic of the main components and operation of a typical LFA. (a) An LF strip is made of four main parts: the sample pad, the conjugate pad, the membrane and the absorbent pad. They are mounted on a laminated card. The membrane binds to the capture bioreceptors that form the test and control lines. Panels b and c show the operation of an LFA based on immunosandwich recognition. (b) The presence of the target analyte in the sample results in the accumulation of nanoparticles on the test and control lines, making the classical two red lines appear. (c) In the absence of the target analyte, the nanoparticles accumulate only on the control line, giving a single colored line output[7].

Lateral flow assay (LFA) design has evolved over the years, 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 specifically 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 glucose testing. For example, the enzyme glucose oxidase converts glucose to gluconic acid and hydrogen peroxide, 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 emit light when excited by a 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 and are often labeled with a tracer such as gold nanoparticles, enzymes, or fluorescent dyes to generate a visible signal. These are some of the common trends in LFAs design, but new technologies and reagents are being developed constantly.

This review presents the LFAs new integration trends of 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 remote and underdeveloped areas and in traditional clinical settings.

2. LFA design and readout systems

2.1 Comparison of different LFAs design

There are many different types of POC lateral flow assay available, each with their own unique design features. The following table compares the features of five popular POC LFAs for the limit of detection (LOD) ranges, analytical sensitivity, and detection times. The conversion of units between g/mL and molarity (M) can be done by calculating the molar weight of the analytes. An electrochemical lateral flow assay (eLFA), which is mainly a biosensor, introduce automated signal detection technique for improving analytical performance in terms of ease of use, rapidity, sensitivity, and selectivity[14]. Chemically enhanced LFAs (Laser-induced Fluorescence Assays) make use of new labels and reagents to improve accuracy[15]. Photothermal LFAs use techniques such as thermal contrast photoacoustic imaging, photothermal laser speckle imaging and thermal

photonic lock-in imaging[16-19]. SERS (Surface-Enhanced Raman Scattering) based PCR (Polymerase Chain Reaction) can also be utilized 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. These MNPs can be detected and measured using external devices, enabling the creation of immunochromatographic tests that have the ability to 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 readout system

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

Table 2. Comparison of 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 good for measuring analyte concentration. To measure concentration, the color of the test zone can be scanned and analyzed with equipment or a smartphone app. However, this method has a narrow working range and is highly nonlinear. Therefore, there is a need to find new labels and readout technologies for LFA. SERS tags are nanoparticles used in various 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 detection of a single tag and proportional measurement of tag concentration. Their narrow spectral bands are

suitable for multiplexing, and studies have shown increased sensitivity and dynamic range in LFA using SERS readout.

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 in multiplex. However, the need for expensive equipment and long signal acquisition time limits its use as a standard immunological technique for point-of-care testing. 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 Raman background and modifying SERS tags to eliminate nonspecific adsorption. Combining SERS-LFA with biochips could lead to a portable and ultrasensitive detection platform for point-of-care 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 used for detecting of Rabbit Immunoglobin G with significantly reduction in assay time without a loss of sensitivity[47]. Similar concept had been used to SERS-based LFAs assay for the quantitative analysis of 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 configuration and the measurement principle of the SERS-based lateral flow assay is shown in **Figure 2.**

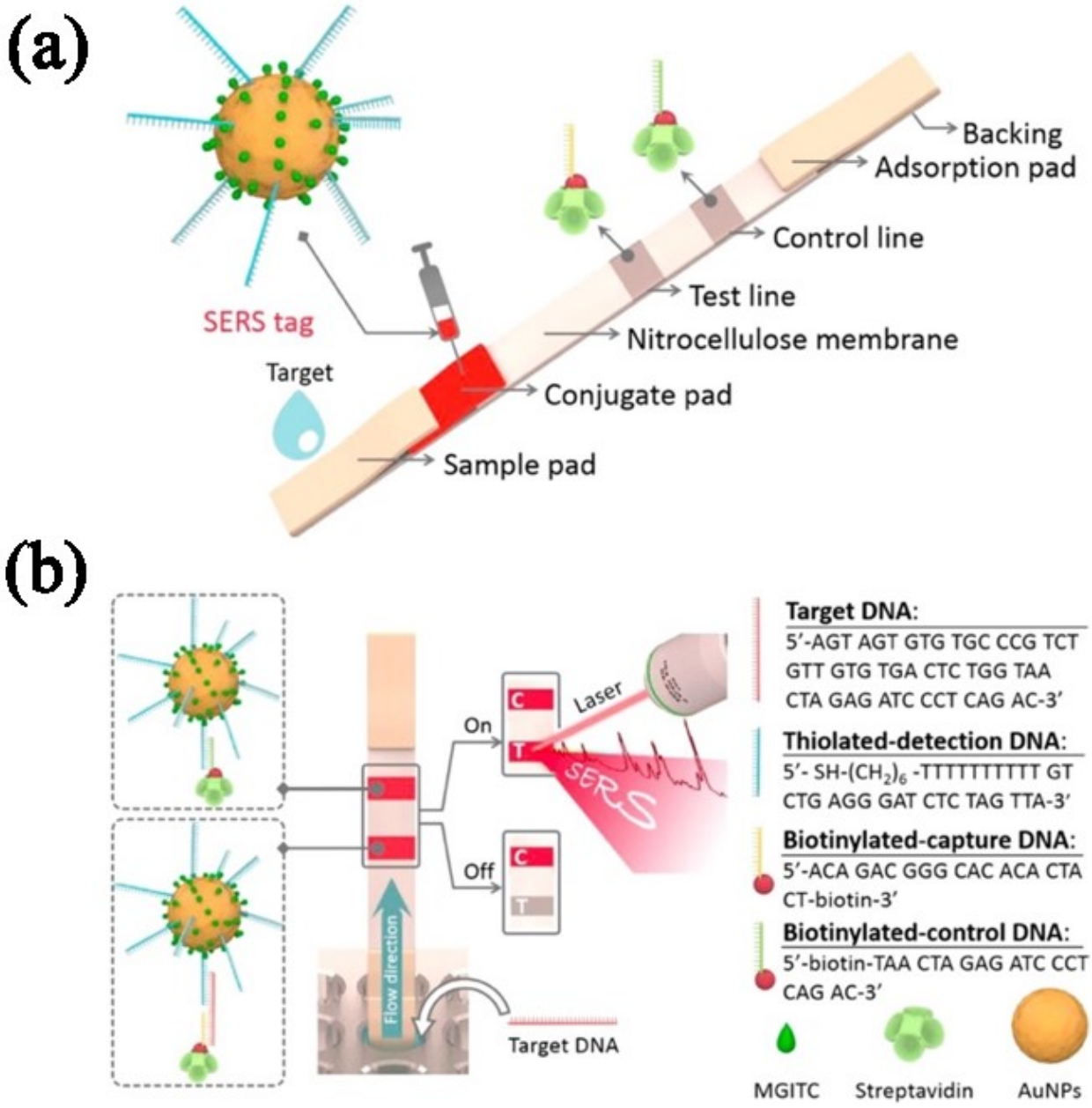

Figure 2. (a) Schematic illustration of the configuration and (b) the measurement principle of the SERS-based lateral flow assay for quantification of HIV-1 DNA. (C is the control line and T is the test line)[48].

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, they enable multiplex detection of multiple analytes in a single sample. Thirdly, they have a wider dynamic range, allowing for accurate measurement of high and low analyte concentrations. Lastly, they are portable and user-friendly, making them ideal for point-of-care testing in resource-limited or remote settings.

Photoacoustic-based lateral flow test was first introduced by Zhao et al. where they demonstrated that uses the strong interaction of light and gold nanoparticles to detect a disease biomarker quantitatively. 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 manner. Since then, photoacoustic point of care testing has evolved to include a variety of measurements, including glucose, cholesterol, uric acid, and other biomarkers. When compared to colorimetric measurements, photoacoustic analysis improved the detection limit of a commercially available lateral flow test strip by two orders of magnitude[17]. The schematic diagram of the LFA paper strip and two PA detection setups is shown in **Figure 3.** The test strip includes nanoparticles that are intended to bind to the target analyte. A photoacoustic sensor detects the sound waves produced when light is glimmered on the test strip after the nanoparticles have absorbed the light. This produces a signal that shows the analyte is present 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. The potential to transition from laboratory research to preclinical and clinical reality is made possible by its integration with existing diagnostic platforms[50].

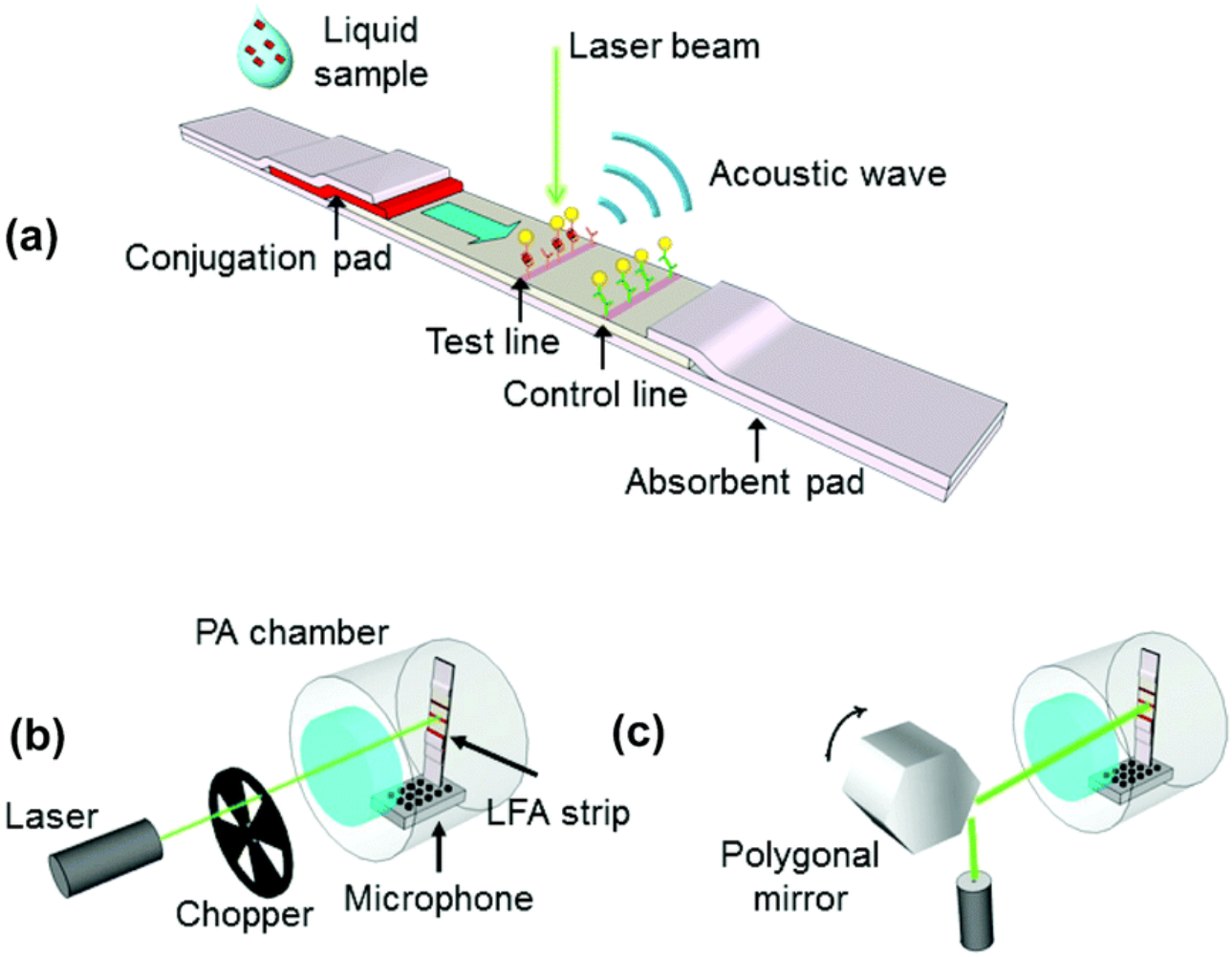

Figure 3. Schematics of the LFA paper strip and two PA detection setups. (a) Shows an LFA paper strip illuminated by a laser beam to generate PA signals. (b) and (c) Illustrate the PA detection systems for the chop mode and the scan mode, respectively[17].

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 wider dynamic range and can be easily automated for high-throughput analysis. These advantages are due to the FRET signal, which is generated only when the target analyte is present, reducing false positives. Further, FRET-based LFA could be used in a variety of sample matrices, including blood, serum, saliva, and urine, making them useful for a wide range of applications.

Wang et al.[51] developed a high-performance fluorescence LFA strip is shown in **Figure 4** for the rapid, sensitive, and specific detection of SARS-CoV-2-specific IgM/IgG in clinical samples by using S protein-conjugated SiO2@DQD nanotags. The unique SiO2@DQD NP was created for use in clinical samples and has excellent luminescence, monodispersed, and good stability. The SARS-CoV-2 spike protein was immobilized onto the surfaces of SiO2@DQD NPs, whereas anti-human IgM and IgG antibodies on the two test lines enabled the simultaneous and highly specific detection of anti-SARS-CoV-2 IgM and IgG in one human sample. By optimization of the assay conditions, the proposed SiO2@DQD-based LFA strip can sensitively and simultaneously detect a low concentration of IgM/IgG (1 : 107 dilution) from 1 μL serum within 15 min. FRET phenomenon also utilizing with LFAs for detecting the H5N2 Influenza virus[52]

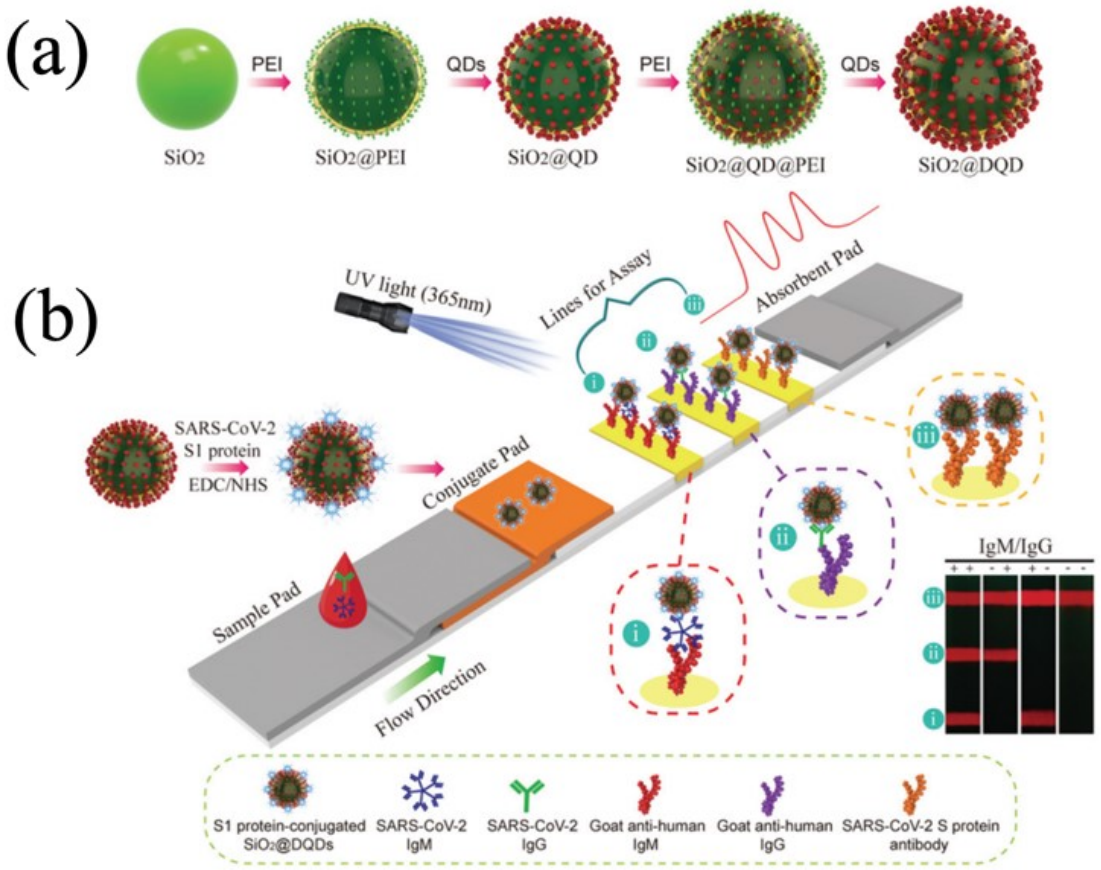

Figure 4. Schematic illustration of the (a) preparation of SiO2@DQD with a dual QD-shell, and (b) principle of the SiO2@DQD-based LFA strip for SARS-CoV-2 IgM-IgG rapid and simultaneous detection[51].

Z. Rong et al.[53] developed an integrated fluorescent LFA platform is shown in **Figure 5** for high-sensitivity detection of HIV antibody, TP antibody, HCV antibody, and HBsAg. The assay was able to detect very low levels of these antibodies, with the lowest detection limit being 0.11 NCU/mL for HIV antibody, 0.62 IU/L for TP antibody, 0.14 NCU/mL for HCV antibody, and 0.22 IU/mL for HBsAg. The test was able to provide results within 20 minutes.

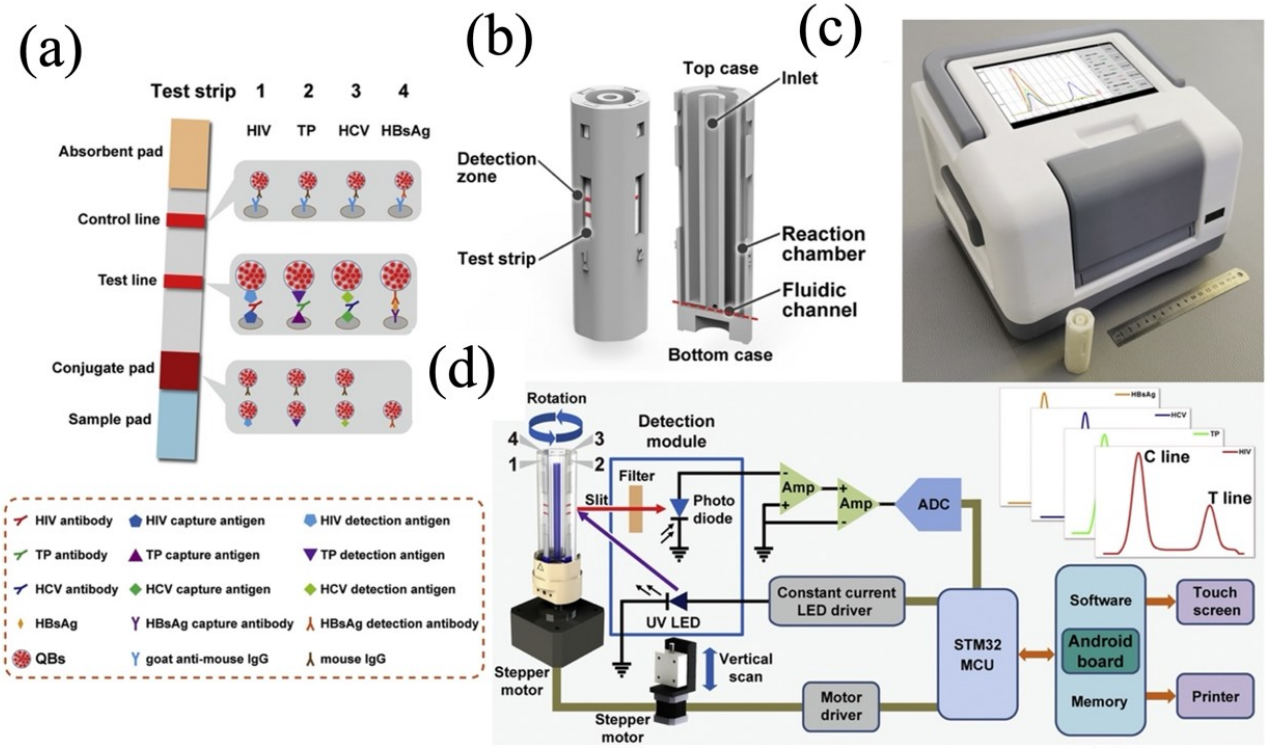

Figure 5. Schematic illustration of the integrated lateral flow assay platform. (a) Illustration of fluorescent lateral flow assay for multiplex detection of infectious disease markers. (b) Design of disposable strip test cartridge. (c) Image of portable strip reader and test cartridge. (d) Principle of optical signal acquisition and analysis[53].

3.4 Thermal contrast reader-based LFAs

The light-to-heat conversion of metallic nanoparticles after exposure to an NIR laser is the basis for the operation of Thermal Contrast Readers [27]. This approach uses thermal contrast amplification (TCA) to improve the sensitivity of the traditional colorimetric LFAs. It utilizes

surface plasmon resonance of gold nano particles under laser irradiation [16]. Here thermophysical and biological responses of aqueous media heated by laser activated GNPs are the main points of investigation [54].

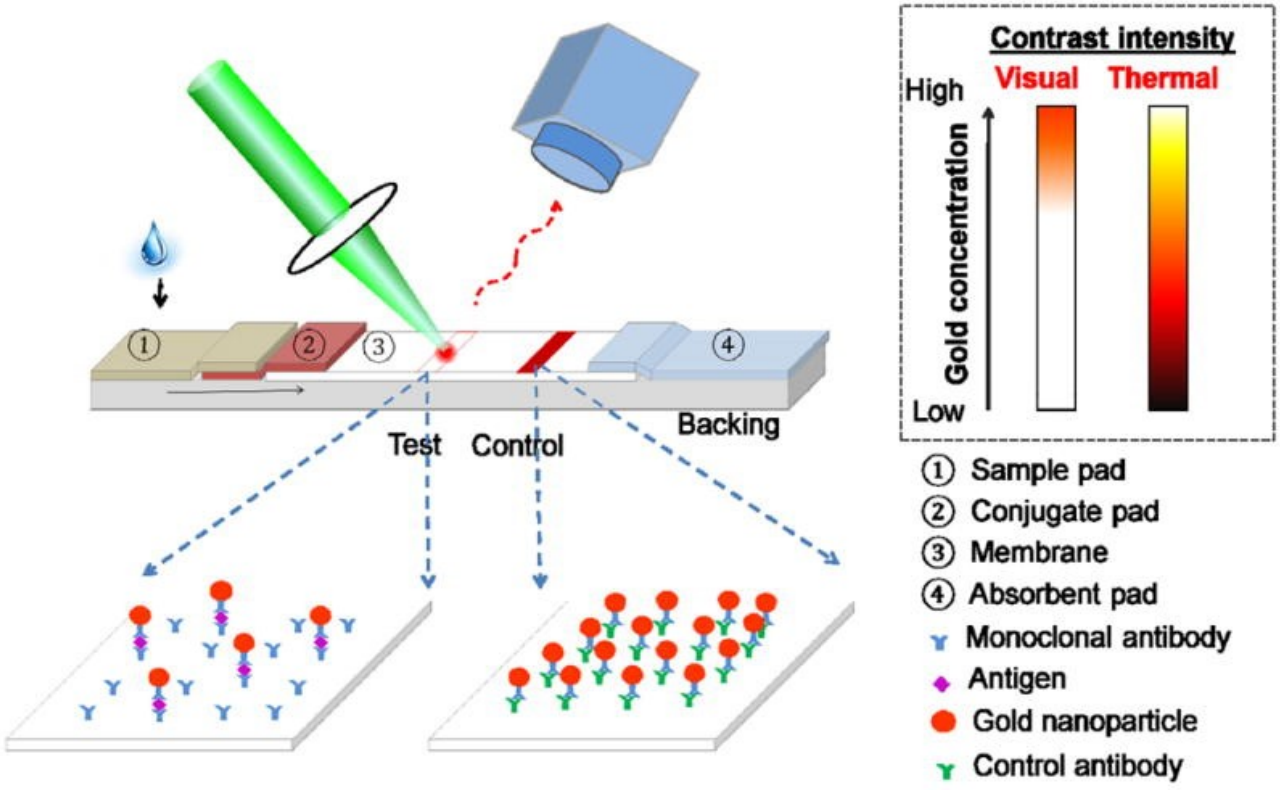

Figure 6. Thermal contrast amplification (TCA) principle and system [55].

According to [56], the TCA reader specifically matches the laser wavelength to the peak of the GNP plasmon resonance, causing heat to be produced and a temperature rise that can be monitored by an infrared detector inside the TCA reader. This heat generation is proportional to the laser intensity and GNP concentration and is connected to the laser wavelength and GNP geometry. As a result, we can accurately identify and measure the GNP signal for a known LFA [56]. In a TCA reader system, there are three main components according to their functions – IR camera, Green Laser, Control. For position and time control LabVIEW can be used, and for sample controlling LFA specific holder is used [16]. For a TCA reader system, data acquisition was the first step in the algorithm's development, followed by data reduction and display in the second stage. An LFA's control line, background, and test line regions—which are reproducibly separated

by defined distances for each manufacturer—are all inspected during one full reading. One example is shown in Figure 7.

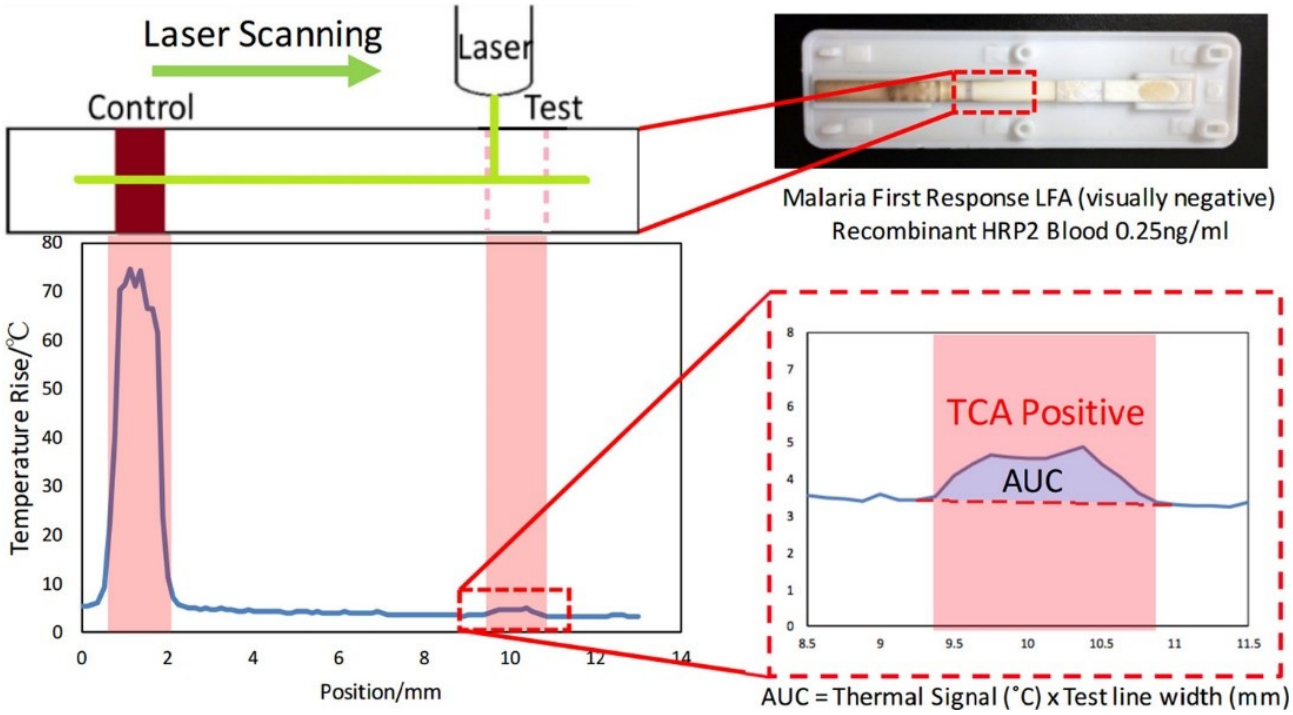

Figure 7. TCA reader algorithm for detection and quantification of temperature rise in an LFA [16].

Wang et al. [16] (Figure 7) showed how an area under the curve (AUC) analysis on the test line position of the LFA can be used to obtain a thermal signal. In this proof of concept, the TCA reader demonstrated that a visually negative malaria First Response LFA was a genuine positive. This LFA's thermal signal score is 1.144 °C as opposed to the negative control average's 0.179 °C (SD = 0.053).

3.5 Magnetic nanoparticles-based LFAs

Magnetic LFAs combines the speed and ease of LFAs with the use of magnetic nanoparticles (MNPs) as markers. MNPs are created particles having a functionalized surface that can particularly bind to target analytes and a magnetic core that is commonly made of iron oxide. Giant magnetoresistance (GMR)[57], tunneling magnetoresistance (TMR), and magnetic particle quantification (MPQ)[58] are examples of magnetic readout techniques. Due to its capacity to recognize the magnetic signal produced by the MNPs, magnetic sensors are frequently utilized in magnetic LFAs. These sensors are able to quantify or qualitatively describe changes in magnetic

field or magnetic characteristics brought on by the presence of MNPs. Nguyen et al. [59]conducted a comprehensive review encompassing these sensors in their study as shown in figure 8. Depending on how neighboring ferromagnetic layers are oriented, the GMR effect, which is seen in multilayers of alternating ferromagnetic and nonmagnetic conductive layers, causes a considerable variation in electrical resistance. The applied magnetic fields interact with the MNPs, altering the measured electric resistance, allowing for the quantification of the MNPs in GMR-based detection for lateral flow tests. Human chorionic gonadotropin (hCG), cardiac troponin I (cTnI), and interferon gamma (IFN- γ) have all been detected using GMR sensors, which have higher sensitivity than 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]. 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 number of biomarkers bound to the MNPs can be quantitatively quantified by monitoring the ensuing resistance changes. Compared to GMR sensors, TMR sensors offer a better magnetoresistance ratio and less field noise. Lei et al. [63]created a prototype for contactless scanning that quantitates MNPs in LFA strips using TMR sensors, demonstrating the detection of hCG at a concentration of 25 mIU/mL.

MPQ sensors measure the nonlinear magnetization response of the particles when they are exposed to a magnetic field in order to quantify magnetic particles. To determine the concentration of the particles, one can measure the induction response the particles produce in an alternating magnetic field. At some combinatorial frequencies, sensitive and reliable measurements can be made by combining two AC frequencies (f1 and f2)[64]. This method enables the exceedingly sensitive scale measurement of relative changes in magnetic susceptibility. For the detection of prostate-specific antigen (PSA), Orlov et al.[65] created an immuno-magnetic MPQ-based LFA detector and showed how highly sensitive and linear it is. 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 gadget has the advantage of monitoring membrane-dwelling particles, which is not possible with traditional optical detection techniques. The MPQ detector has also been used in

multiplexed detection for the quantitative multiplexed detection of cardiac biomarkers and the discriminating of several botulinum neurotoxins.

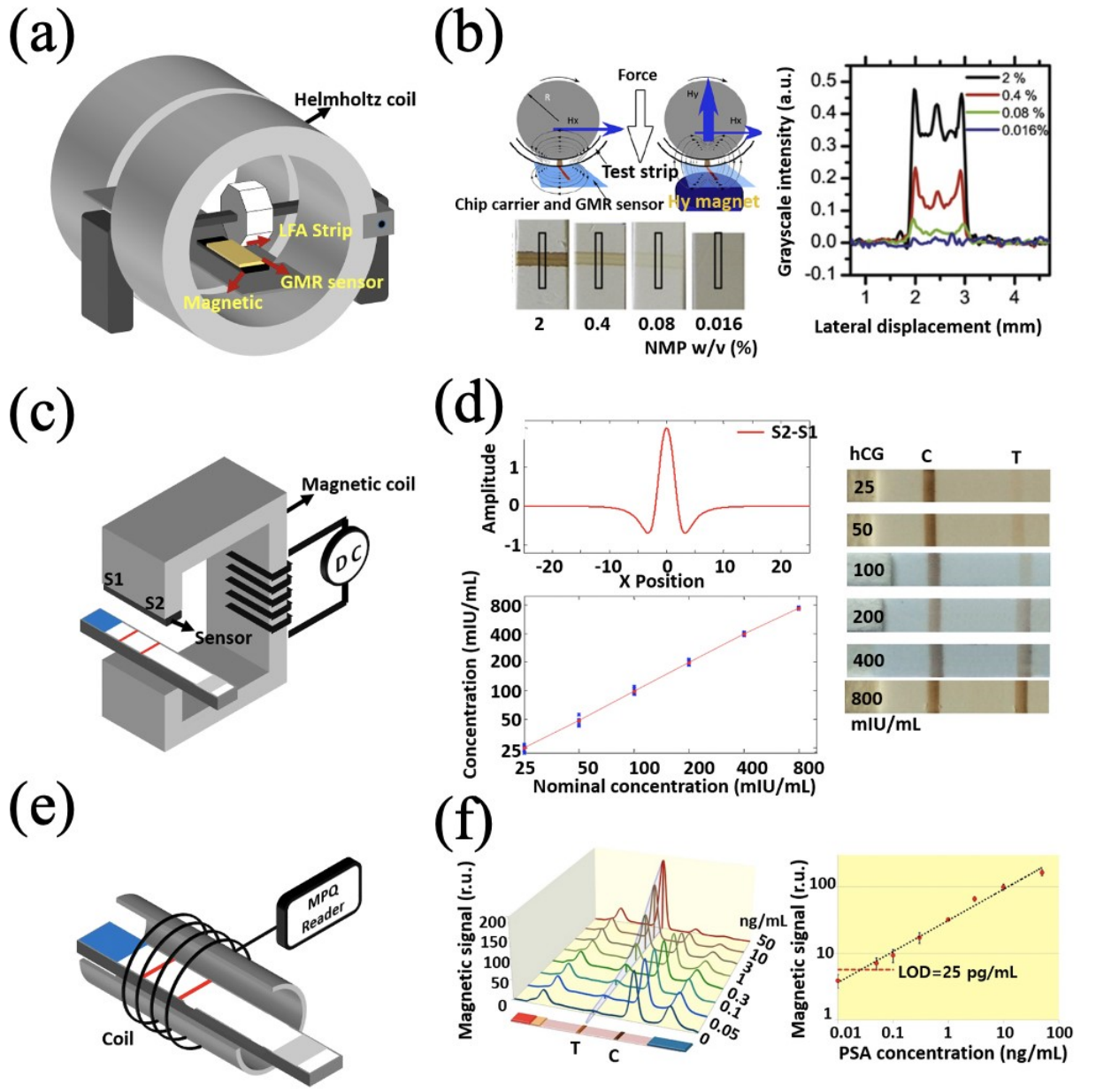

Figure 8. High-sensitivity LFAs based on the magnetic responses of tracing particles. (a, c, e) Schematics for GMR, TMR, and MPQ based LFAs. (b, d, and f) are the corresponding representative applications of a, c, and e, respectively[59].

3.6 Smartphone-based LFAs

Smartphone-based lateral flow assay (SLFA) has a significant effect on many different types of point-of-care medical diagnostics. Infectious disorders, cardiac and tumor indicators, other hormones, or medication screening may all be readily included into this system using currently available, FDA-approved LFAs test strips[66]. Gong et al.[35] developed an up-conversion nanoparticle-based lateral flow assays (UCNP-LFAs) as shown in figure 9.

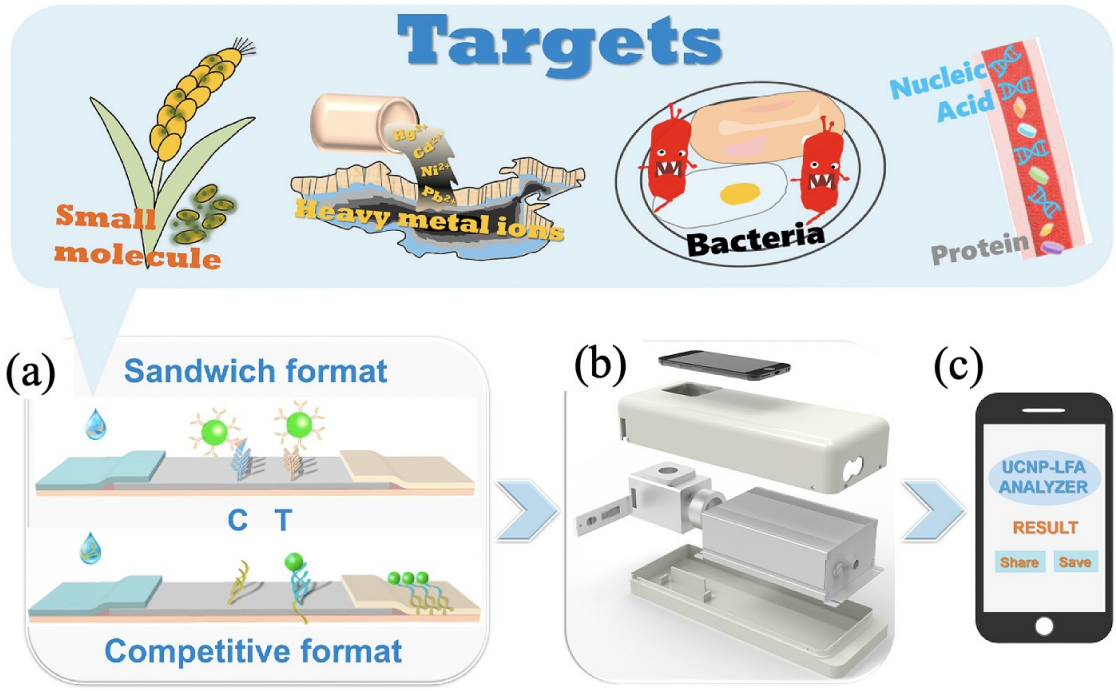

Figure 9. Schematic diagram of the UCNP-LFA platform. (A) LFA detection system; (B) UCNP-LFA reader and (C) UCNP-LFA App [35].

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 depending on color hues and lighting conditions, these smartphone readers can easily detect that test band. Smartphones can capture a digital image of the experiment's outcome with time and date stamps, which is useful for database purposes. A smartphone with a built-in camera was utilized by You et al. [66] to read TSH levels. However, since the high-density charge-coupled device (CCD) or complementary metal oxide semiconductor (CMOS) arrays used in cell phone cameras are not ideal for extremely high sensitivity applications, they made use of the Mie scatter to optimize the system's parameters in order to boost the sensitivity, repeatability, and usability of

the TSH LFA. They showed how to employ low-cost point-of-care equipment, Figure 10, to test extremely low quantities of TSH (0.5 mIU L^{-1}), using the technology of the optimized Mie scatter detection on a nitrocellulose LFA. This makes it possible to identify hyperthyroid conditions, which otherwise need a clinical laboratory. With an assay time of roughly 12 minutes and a detection limit of 0.31 mIU L^{-1}, the gadget demonstrated good sensitivity and repeatability.

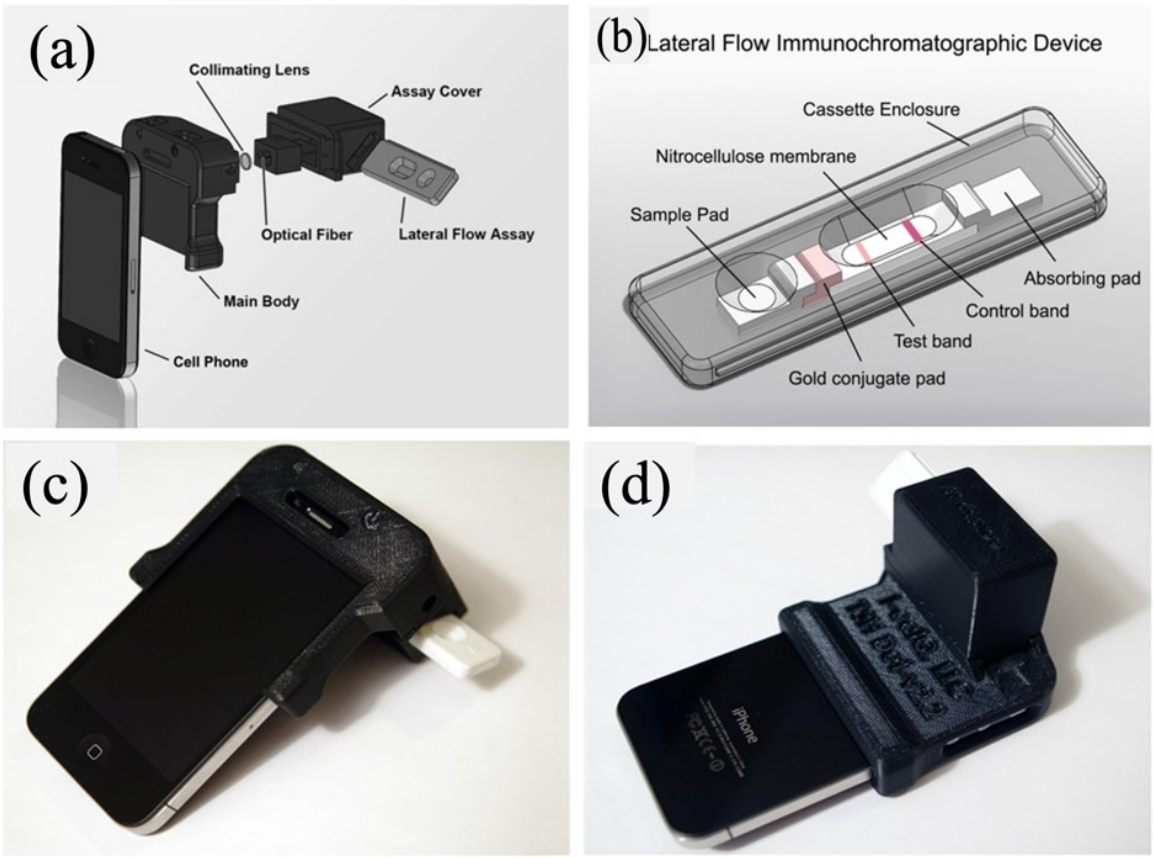

Figure 10. (a) Exploded view of complete device showing placement of collimating lens and optical fiber (light pipe) set at specific angles in reference to the lateral flow assay cassette. (b) Cheap and disposable lateral flow assay test strip, with gold nanoparticle conjugated antibodies in the gold conjugate pad, anti-TSH immobilized in the test band, and anti-IgG immobilized in the control band. Flow is from left to right. (c, d) Photographs of actual reader attached to cell phone and with a disposable TSH LFA cassette inserted[66].

Rong et al.[68] developed a smartphone-based fluorescent LFIAs platform for the highly sensitive point-of-care detection of Zika virus nonstructural protein 1 (ZIKV NS1). The study showed that how a 3D-printed attachment can integrate external optical and electrical components

with a smartphone to enable downsizing and cost savings. With this method, ZIKV NS1 quantitatively detected at the point of care in under 20 minutes, LODs were 0.045 ng mL-1 for buffer and 0.15 ng mL-1 for serum, respectively. Figure 11 represents the schematic of the smartphone-based imaging device. This apparatus used a high-power UV LED (5 W) with emission at 365 nm as the QD microspheres caught on the LFIA strips' excitation light source. To lessen the background noise by eliminating the directly reflected excitation light, the LED was fixed to a module with a 60 degree incidence angle and 20 mm between it and the LFIA strips. The power unit included a switch, a constant current drive circuit, and a lithium battery with a two-hour continuous runtime. By reducing the object distance from around 80 mm to 20 mm, the external plano-convex lens installed in front of the camera allows for a more compact design of the overall device structure and the collection of numerous image signals to enhance the signal-to-noise ratio (SNR). To further minimize background noise, a short-pass filter (425 nm) and bandpass filter (624/40 nm) were put in front of the UV LED and external lens, respectively.

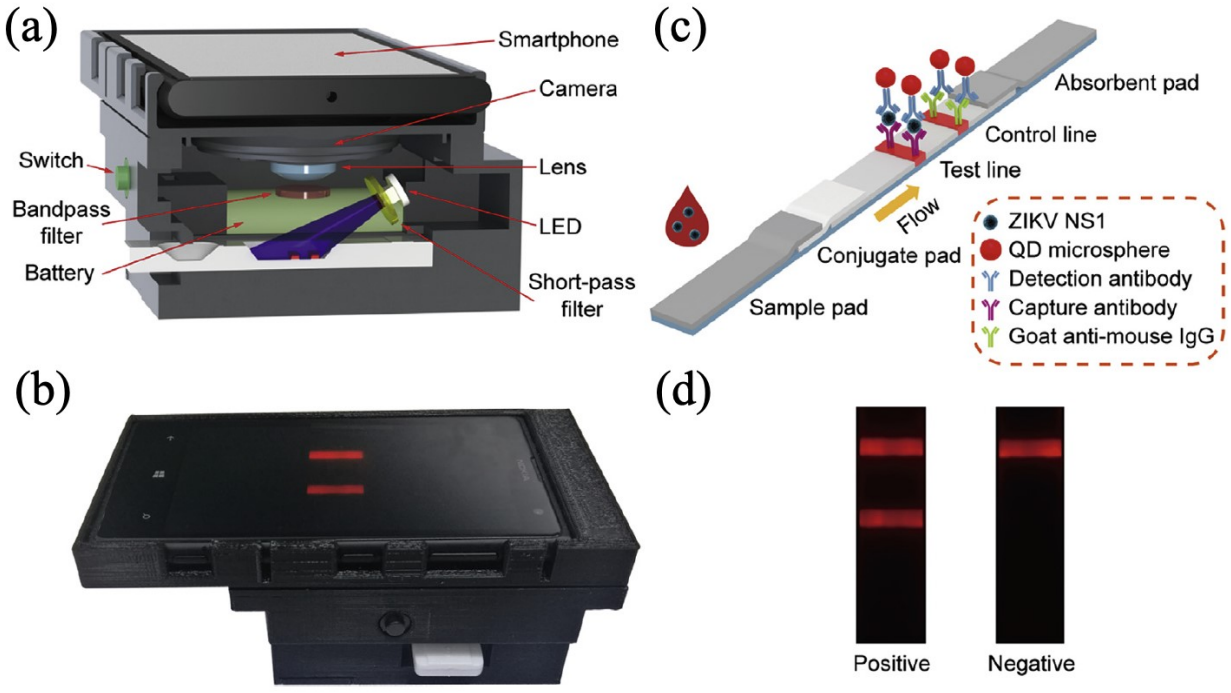

Figure 11. Overview of the design and application of the smartphone-based fluorescent LFIA platform. (A) 3D schematic of the smartphone-based imaging device, showing the internal structure. (B) Photograph of the developed fluorescent LFIA reader. (C) Schematic of the fluorescent LFIA for the detection of ZIKV NS1. (D) Images of the test strips in the presence (left) and absence (right) of ZIKV NS1[68].

Ross et al.[69] demonstrated an Surface Plasmon Resonance (SPR) based approach for screening and choosing crude anti-hazelnut antibodies based on their relative association rates, cross reactivity, and sandwich pairing capacities for subsequent use in a fast ligand binding test. These LFAs not only provide a qualitative result when read visually, but also generate semi-quantitative data when exploiting smartphone apps. Figure 12 shows semi-quantitative smartphone based SPR biosensors.

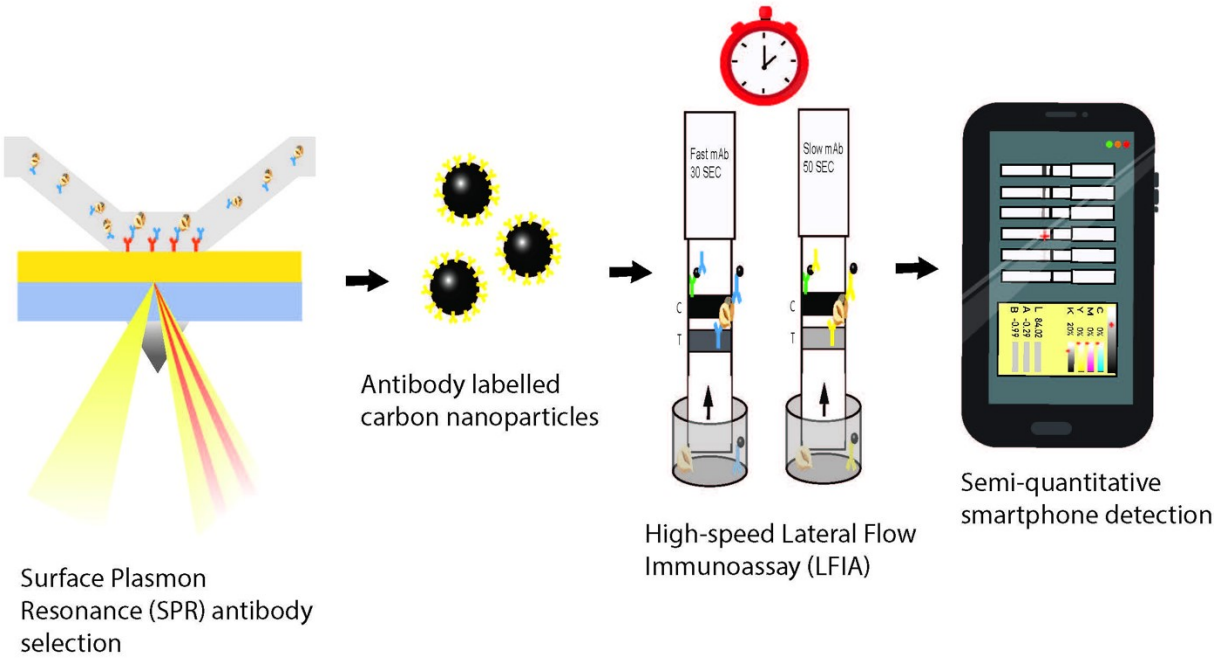

Figure 12. Schematic Overview of Smartphone Based Surface Plasmon Resonance Biosensor [69].

Exploiting smartphones' capacity for SERS detection is extremely important 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 to the back of a smartphone as shown in figure 13, through the use of slit coupling and rational optical path design. It's significant because they could use the smartphone to gather and transmit Raman data to the could over wireless network (for instance, WIFI, Bluetooth, and 4G). The obtained Raman spectrum was then identified using a matching technique and the existing spectrum database. Raman spectrum capture took less than 2 seconds altogether, and wireless communication decreased the whole cost to below $10,000. When utilizing a standard laboratory Raman spectrometer, this is at least ten times lower than the previous measurement. The increased

POCT applications of SERS-based test strips are promised by the smartphone Raman system and potential network architecture[70].

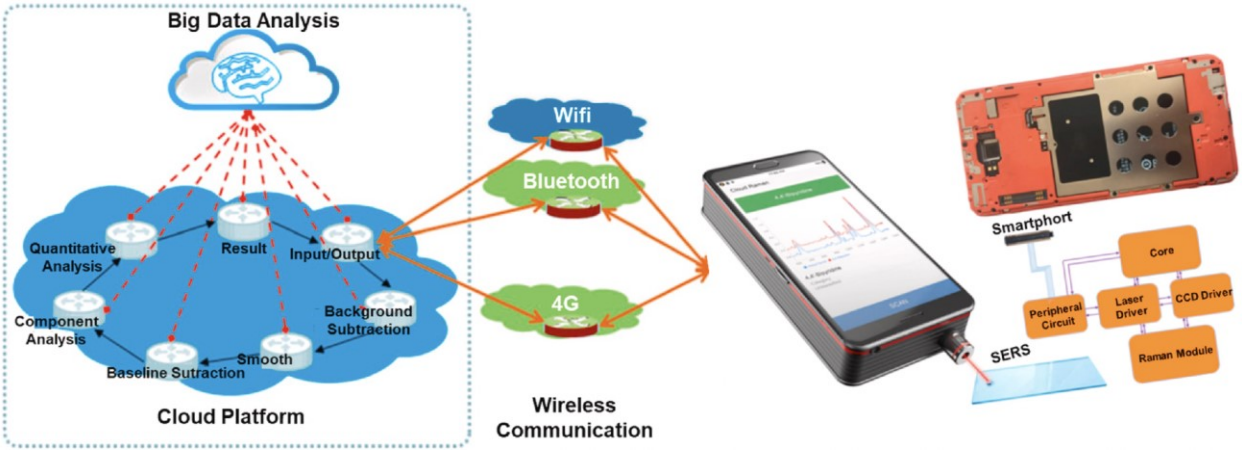

Figure 13. Smart-phone based Raman material identification system based on cloud network architecture [70].

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. There is rising demand for multiplexed systems that can detect many biomarkers at once due to the complexity of human disease, overlapping symptoms, and co-infection states. Multiplexing may only be possible with a few biomarkers because of inherent restrictions in the design of conventional membrane based LFAs. 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 of LFAs, some of the most common are discussed in this review. One of the ways to increase the sensitivity is multiplexing which allows for the simultaneous detection of multiple analytes in a single sample as shown in figure 14. This can increase the sensitivity of the assay by detecting multiple markers of a disease or infection. Multiplexing on a single stirp has been studied for detecting the Dengue/chikungunya virus (IgG/IgM)[74], Antibodies against HIV-1 and -2, Mycobacterium[75], Dengue virus NS1 protein, Yellow Fever Virus NS1 protein, and Ebola virus, Zaire strain glycoprotein GP[76], Anti-HIV IgG, Anti-HCV IgG, Anti-HAV IgG and IgM[77]. Multiplexing with multiple strips has been studied E. coli O157:H7, S. paratyphoid A, S. paratyphoid B, S.

paratyphoid C, S. typhi, S. enteritidis, S. chlorosis, V. cholera O1, V. cholera O139, 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].

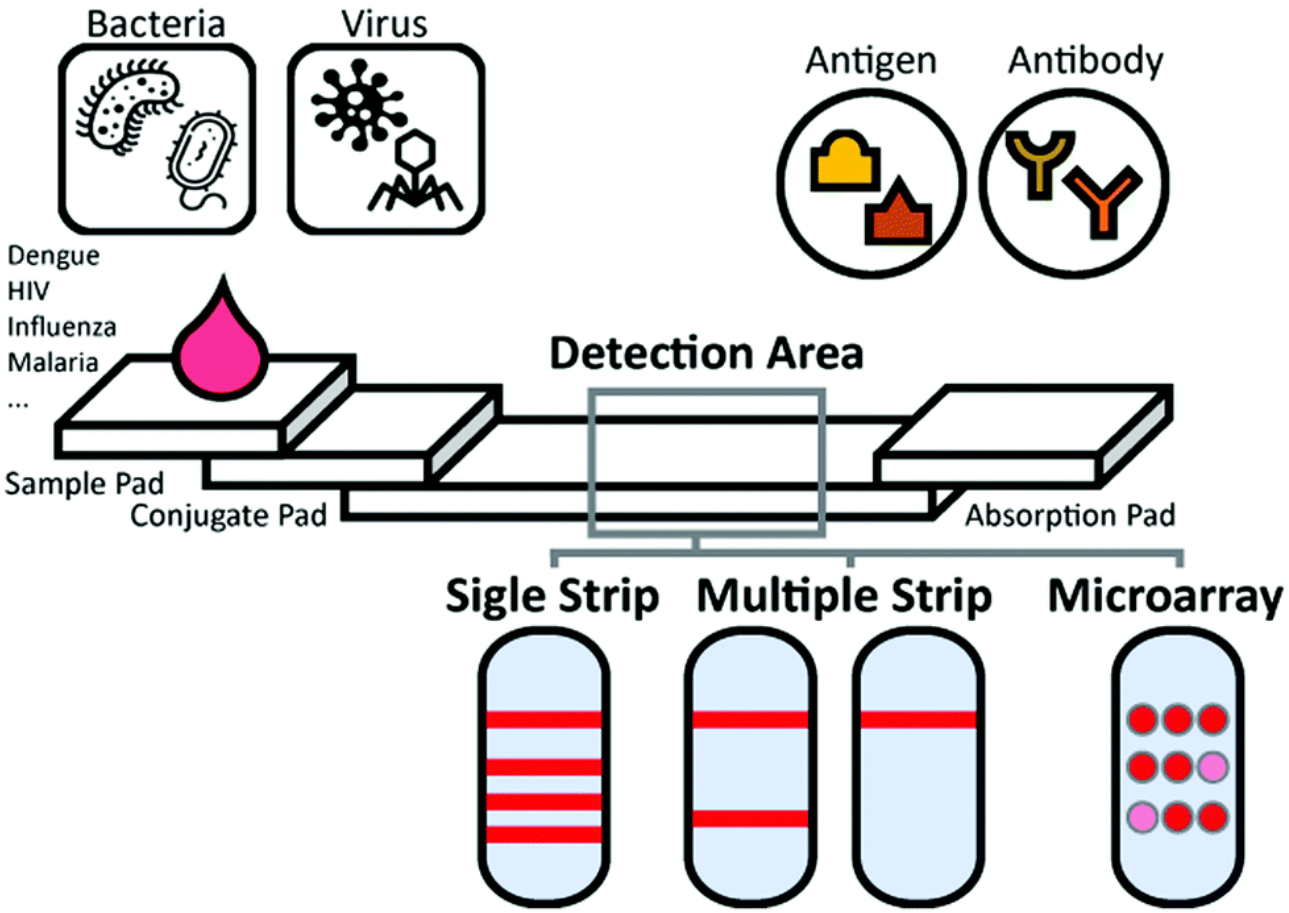

Figure 14. Schematic of multiplex lateral flow immunoassay targeting infectious disease-causing viral agents[81].

Different nanoparticles can also increase the sensitivity of the assay by amplifying the signal generated through the binding of the analyte to the test line as shown in figure 15. The most frequently used labeling agents in LFAs are antibodies combined with nanoparticles like magnetic nanoparticles[82], carbon-based nanoparticles[83], silver nanoparticles[84], quantum dots[85], fluorescent dyes, and up-converting[86] phosphor. Gold nanoparticles are the most popular of these and are widely employed for biosensing due to the 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 used for the detection of non-immunogenic and toxic targets. For instance, Shim et al.,[89] developed an aptamer-based LFAs for the rapid and easy detection of aflatoxin B1. A specific aptamer for aflatoxin B1 was labeled with the fluorescent material cyanine 5 (cy5) for optical identification. Through optimization, the dipstick assay achieved a LOD of 0.1 ng/mL. Fu et al., [48] developed a highly sensitive lateral flow immunoassay method for the specific detection of thrombin in clinical analysis. This method utilized gold nanoparticles (AuNPs) and a pair of aptamer probes to achieve enhanced sensitivity. However, higher cost associated with gold nanoparticles (AuNPs), researchers have explored the use of common dyes, such as blue dye, as alternative labels for protein detection on the LFAs platform[90]. Zhao et al.,[91] reviewed how point-of-care applications demonstrate the pairing of highly specific recognition between aptamers/antibodies and targets with the exceptional features of a dry-reagent strip biosensor, enabling effective detection. Mao et al.,[92] proposed a quantitative lateral flow aptamer strip sensor utilizing blue dye-doped latex beads (modified with carboxyl groups). In this approach, blue dye-doped latex beads and aptamer conjugates were captured on the test line in the presence of target DNA, resulting in the formation of a visible blue band. Through optimization, the strip assay achieved a limit of detection (LOD) of 3.75 fmol/L synthesized DNA in human plasma samples. Lee et al.,[74] presented a novel assay scheme that employed two-color latex labels for the rapid detection of acute febrile illnesses (AFIs).

mRNA (messenger RNA) an alternative reagent has gained significant attention in recent years, particularly in the field of 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 are made possible by the capture and detection of mRNA targets on the test line using complementary probes or primers. Similarly, DNA-based reagents LFAs are used to find certain 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 certain genetic variants linked to diseases[98]. LFAs may also contain synthetic polymers to enhance the performance of the assay[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]. Another reagent enzymes are essential components in many LFAs as they provide signal amplification[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 (HRP) and alkaline phosphatase (ALP) are two common enzymes in LFAs[103]. These enzymes have the ability to 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 specific requirements of the assay, including the nature of the target analyte, 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].

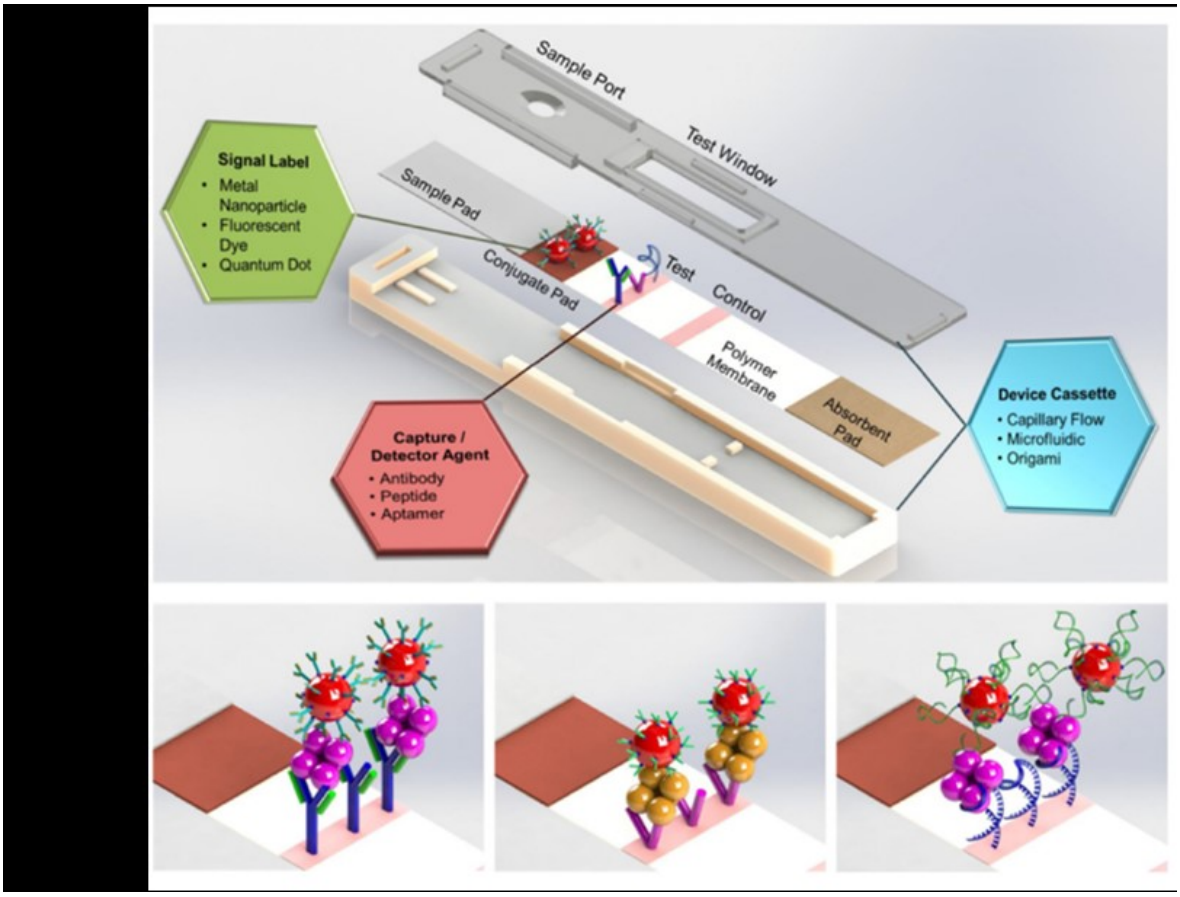

Figure 15. (a) Schematic depicting the general layout of a LFA test kit, as well as components (in colored boxes) of the test kit that can be further developed and optimized. (b) Sandwich complex formed when the

target molecule binds to the capture and detector agents, composed of antibodies (left), peptides (center), and aptamers (right), at the test line[107].

Researchers are also exploring the use of signal amplification techniques, such as enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR), to increase the sensitivity of LFAs [108]. S.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].

There is some research being pursued on label-free detection methods and these are typically based on the measurement of changes 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 assay. X. 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) quickly and sensitively. This method does not require labels and instead uses high luminescent quantum dots (QDs) as the signal output. A study was conducted that revealed a new label-free and dual-readout LFIA (LD-LFIA) that was mediated by a "Three-To-One" multi-functional nanocomposite. 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]. B. Tasbasi et al.[112] found that biosensors based on aptamer-gated silica nanoparticles have been reported for their high levels of sensitivity, specificity, and label-free detection of small molecules and whole cells is shown in figure 16. This label-free approach to detection of Listeria monocytogenes can be used to accurately determine its presence. Finally, the integration of these technologies has allowed for more sophisticated data analysis, providing better insights into the causes of diseases and conditions.

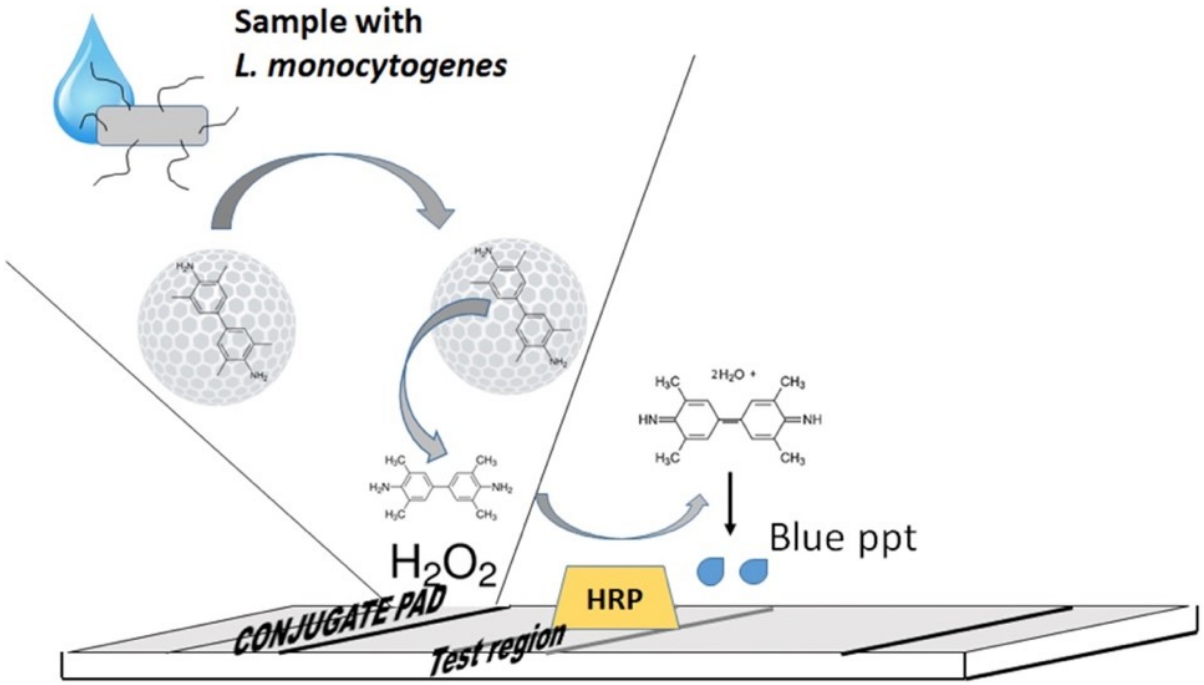

Figure 16. Schematic representation of assay principle. L. monocytogenes cells migrate to the conjugation pad region where binding to aptamer gates. TMB from nanopore and converted to blue precipitates by peroxidase immobilized at the test region[112].

4.1 Sensitivity enhancement strategy

Lateral flow assay devices are popular for medical diagnostics but their sensitivity is limited which 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 limit of detection and accurately detect low levels of analytes. Two alternative formats of enzyme-linked immunosorbent assays that can be converted to LFAs are sandwich LFAs and competitive LFAs. Despite the fact that each style has benefits and drawbacks, sandwich LFAs are more frequently employed than competitive LFAs[113]. The target analyte is caught in a sandwich-like LFA between two distinct antibodies, frequently referred to as the capture antibody and the detection antibody. The target analyte in the sample is bound by the capture antibody. The detection antibody then attaches to a separate epitope on the target analyte, creating a "sandwich" complex[8]. The detection antibody is tagged with an enzyme or another detectable marker. The target analyte can be quantified or seen thanks to the enzyme-linked detection antibody. 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 is appropriate for both qualitative and quantitative assessments since it can cover a broad range of analyte concentrations. Different capture and detection antibody combinations can simultaneously detect a number of 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. In a competitive LFA, a labeled analyte (commonly called the tracer) competes with an analyte present in the sample for binding to a little quantity of immobilized capture antibody. Less tagged analyte will bind to the capture antibody as more analyte is present in the sample[8]. The amount of analyte in the sample has an inverse relationship with the signal that is detected. Since the labeled analyte competes with the analyte in the sample for binding to the capture antibody, competitive LFAs often has lesser sensitivity compared to sandwich LFAs[115]. Accurately detecting low amounts of the target analyte may become more difficult as a result. Competitive LFAs is better suited for qualitative or semi-quantitative measures rather than accurate quantification because it has a more constrained dynamic range[116]. The competitive format necessitates the optimization of a number of 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 as a result of this intricacy. Considering the higher sensitivity, wider dynamic range, and simpler assay design, the sandwich LFAs design is generally preferred over the competitive LFAs design. However, it's worth noting that the choice of LFAs format depends on the specific requirements of the assay, 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, the simultaneous analysis of several analytes in a single sample, is a useful tool for diagnostic tests as it can measure multiple biomarkers indicative of disease progression. This can inform clinical decisions based on the needs of individual patients. The utility of multiplexing is extended to food, environmental, and safety applications, where the assay's power increases with the number of targets detected. Multiplexing is also beneficial in saving sample volume, time, and cost, as most diagnostic samples are extracted in limited volumes[27]. However, the widespread use of multiplexing in LFAs is hampered by a number of 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 issue has been observed in various infectious diseases like flavivirus infections (such as dengue and Zika virus) and helminthic infections (like strongyloidiasis and filariasis). Interestingly, these infections often exhibit overlapping symptoms, further complicating the accurate detection and differentiation of

specific biomarkers in multiplexed LFAs [127, 128]. The quantity and variety of biomarkers that can be included in a single LFAs are constrained by this restriction. Physical restrictions are also a factor, as multiplexing many test lines on a single LFAs strip is limited by the quantity of conjugates and the variations in fluid flow while passing over several lines [129]. Additionally, selecting the patient and control populations, using calibration techniques, and validating processes are only a few of the operational and quality assurance difficulties that arise when converting existing assay assessment designs for single-biomarker LFAs to multiplexed LFAs. The interpretation of numerous test findings can add complexity, thereby undermining the LFAs simplicity. The sharing and teamwork necessary for generating multiplexed LFAs are made more difficult by the proprietary nature of many proprietary biomarkers employed in single-test LFAs. The translation of multiplexed LFAs into commercial devices is still limited, despite the development of promising prototypes in research [130], underscoring the necessity of addressing these issues through more research and development efforts. The necessity for multiplexed LFAs, significant technical and operational problems for multiplexing, inherent in the design and production of multiplexed LFAs, as well as newly developing enabling technologies that may be able to overcome these challenges, were all outlined by Khayriyyah et al [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 multiplex test with different viral antigens for the diagnosis of AIDS, hepatitis C and A (HCV, HAV), and HCV [77]. By enabling the simultaneous detection of several targets, these extraordinary developments in multiplexed LFAs emphasize the potential for increased diagnostic capabilities, which will lead 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	glutamate dehydrogenase (GDH) and Clostridioides difficile toxins A and B	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 for the diagnosis of 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 different metrices relevant to LFAs applied to detect various infectious 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 difficult 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 temperature, humidity, and light can also affect the stability of new components, which can pose 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 a lengthy and costly process. 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 operation is another important challenge that can arise when incorporating new technologies and reagents into LFA design[199]. As LFAs become more complex, they may require extensive user training to ensure proper assay performance. 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, which can increase the complexity and time required to perform the assay, leading to increased costs and reduced throughput. Interpretation of assay results may also become more complex with the integration of 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. Overall, addressing the challenge of complexity of operation is critical for successful implementation of 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 present 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 for 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 necessary 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. Overall, addressing the challenge of potential false positives is critical for ensuring accurate and reliable results in LFA design.

6. Conclusion

New technologies and reagents have the potential to improve the accuracy and sensitivity of lateral flow assays (LFAs). Surface Enhanced Raman spectroscopy (SERS) and Photoacoustic techniques can improve the sensitivity of LFAs, while Fluorescence Resonance Energy Transfer (FRET) can enhance specificity. The integration of 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 achieving sufficient sensitivity, 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. Overall, the integration of new technologies and reagents in LFA design requires careful consideration of the benefits and challenges to ensure that the assay is effective, reliable, and safe for use in clinical settings.

The development of new technologies has allowed for significant improvements in lateral flow assays (LFAs), making them increasingly reliable and accurate. In the future, LFAs are expected to become even more accessible and efficient, with greater accuracy and sensitivity. Rapid and accurate results are becoming increasingly demanded, and LFAs will become more reliable, reducing the time needed to get an accurate result[207]. The sensitivity of LFAs is expected to increase, allowing for the detection of smaller amounts of the target analyte, which could be used for various applications such as food testing, water quality monitoring, and environmental testing. The focus on consumer needs is also driving LFA development, with the technology becoming more user-friendly, allowing for easier testing by untrained individuals[208]. The cost of LFAs is expected to decrease, making them a viable option for different uses. Overall, the future of LFA design and testing looks promising, and with increased reliability, LFAs could become valuable tools for healthcare professionals to diagnose different conditions quickly and accurately.

Continued innovation in the field of LFAs technology is significant because it allows for the development of more accurate, sensitive, and user-friendly diagnostic tools. LFAs have already revolutionized healthcare by providing quick and affordable tests for a variety of diseases and conditions. However, there is still a lot of room for improvement. For example, current LFAs may not be sensitive enough to detect low levels of certain analytes, which can lead to false negatives[209]. Additionally, some LFAs require trained personnel to operate and interpret the results, which can limit their use in resource-limited settings. Innovations in LFAs technology can address these issues by increasing the sensitivity of the tests, making them more user-friendly, and expanding their range of applications. For example, advancements in nanotechnology and microfluidics can enable the development of more sensitive LFAs that can detect low levels of analytes[210]. Incorporating artificial intelligence and machine learning can help automate the interpretation of test results, making LFAs more accessible to untrained personnel[211].

Innovation in LFAs technology also has broader implications beyond healthcare. LFAs can be used for a variety of applications, such as environmental monitoring, food safety testing, and forensic analysis[212]. By improving the accuracy and sensitivity of LFAs, they can become more widely adopted in these areas, leading to safer environments, more reliable food testing, and more effective forensic investigations.

Overall, continued innovation in the field of LFAs technology is crucial for improving the accuracy, sensitivity, and accessibility of diagnostic tools, expanding their range of applications, and ultimately improving human health and well-being.

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