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Review

Lateral flow immunoassays for antigens, antibodies and haptens detection

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ABSTRACT

Lateral flow immunoassay (LFIA) is widely used as a rapid point-of-care testing (POCT) technique in food safety, veterinary and clinical detection on account of the accessible, fast and low-cost characteristics. After the outbreak of the coronavirus disease 2019 (COVID-19), different types of LFIAs have attracted considerable interest because of their ability of providing immediate diagnosis directly to users, thereby effectively controlling the outbreak. Based on the introduction of the principles and key components of LFIAs, this review focuses on the major detection formats of LFIAs for antigens, antibodies and haptens. With the rapid innovation of detection technologies, new trends of novel labels, multiplex and digital assays are increasingly integrated with LFIAs. Therefore, this review will also introduce the development of new trends of LFIAs as well as its future perspectives.

1. Introduction

Lateral flow immunoassays (LFIAs) develop on the basis of monoclonal antibody (mAb) technologies, immunochromatography technologies, new materials and labeling technologies [1,2]. LFIAs can realize qualitative and semi-quantitative detection of various analytes such as antigens, antibodies and haptens without professional skills and expensive instruments [3–6]. The LFIA is one of the ideal immune rapid detection technologies, which is widely used in rapid detection of hormones [7], pathogenic microorganisms [8,9], veterinary drugs [8], pesticides [10], biotoxins and other targets [11,12]. LFIAs show broad application prospects in on-site real-time detection, which are especially suitable for hospitals, veterinary clinics, farms, dairies and other fields [13–15].

LFIAs were derived from the latex agglutination test established by Plotz and Singer [16], it is in the same period of time as radioimmunoassay and enzyme immunoassay. The early development and application of LFIA was the determination of human chorionic gonadotropin in pregnant women urine and serum/plasma in the late 1980s. LFIAs achieved the long-sought standard of "assurance" in diagnostic technology (affordable, sensitive, specific, user-friendly, fast and robust, no equipment required), which significantly promoted the development of immune diagnostic technology [17].

According to the different detection formats, LFIAs can be divided into direct detection and competitive detection. The direct detection can be used for the detection of antigens (such as human or animal proteins and pathogenic microorganism proteins, etc.) and antibodies (such as immunoglobulins). It is mostly applied in the early diagnosis of human and animal diseases, biochemical analysis and antibody titer monitoring. The competitive detection is a competitive inhibitory immunological binding reaction and mainly used for the detection of small molecule compounds with few antigenic sites or only a single antigenic site. These small molecule compounds are usually reactogenic but not immunogenic, they are also called haptens. Therefore, competitive detection is of great convenience in testing residues of pesticide, veterinary drug or mycotoxin.

With the rapid innovation of diagnostics, extensive efforts have been invested into carrying out novel labels to improve the sensitivity,

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multiplex detection to realize simultaneous detection of multiple targets and digital detection to realize visual reading and quantitative detection [18,19]. Hence, new trends of new labels, multiplex assays and digital assays are introduced. Finally, the future perspectives of LFIAs are also discussed.

2. Principles and components of the LFIAs

The test strip is the commonest form of LFIA, it consists of four basic structures: sample pad, conjugate pad, nitrocellulose (NC) membrane and absorption pad [1,20], which are stacked on a support plate in sequence from the test end to the handle end (Fig. 1). The sample pad absorbs the sample solution and makes it flow laterally to the conjugate pad by capillary force. The conjugate pad is labeled with bioactive materials (such as colloidal gold-labeled antibodies), which can bind to the target from sample solution to generate immunological complexes. The NC membrane intercepts labeled immune complexes, and visually displays the result. Two or more different biologically active materials (such as antigens or antibodies) are immobilized to form a "test line" (T line) and a "control line" (C line) on the NC membrane. The absorption pad absorbs the sample solution flowing through the strip, which maintains the pressure difference between the two ends and promotes more sample solution to laterally flow on the NC membrane.

3. LFIAs for antigens detection

Sandwich LFIA based on the principle of two different antibodies bind to antigen simultaneously is mainly detecting antigen with multiple antigenic sites, such as pathogenic bacteria, viruses and proteins. Most natural antigen molecules have complex structures with multiple antigenic epitopes on the surface. They can bind to different antibodies simultaneously and be used as detection targets. There are three LFIA formats for antigen detection.

3.1. MAb labeling-polyclonal antibody capture format

The mAb labeling-polyclonal antibody (pAb) capture format is to prepare the conjugate pad by labeling the mAb that specifically recognizes the analyte with nanomaterial. PAb works as a capture antibody for immunological complexes, anti-mouse IgG antibody or *Staphylococcal* protein A (SPA) has the ability to bind immunoglobulin and work as the control antibody. They are coated on the NC membrane as the T line and the C line, respectively (Fig. 2a). During detection, the analyte in the sample is combined with the labeled antibody to form the immunological complexes, which are specifically recognized by the capture antibody, presenting a colored band at the T line. The excess labeled antibody and part of the immunological complexes are recognized by the control antibody, forming the C line, which gives a positive result. In addition, the color intensity is correlated with antigen concentration.

An immunochromatographic strip for the detection of avian avulavirus 1 (Newcastle disease virus, NDV) based on one anti-NDV mAb and one pAb was established by Li et al. in Key Laboratory of Animal Immunology (KLAI) of Henan Academy of Agricultural Sciences (HAAS) [21]. The strip can detect NDV with a detection limit of $10^{4.9}$ EID_{50} viruses/0.1 mL in the NDV infected sample, whose performance is as good as hemagglutinin (HA) test (Fig. 3a). This strip can detect NDV in infected tissue as early as 36 h, before clinical symptoms and gross anatomical lesions.

3.2. MAb labeling-mAb capture format

The mAb recognizes specific epitope of antigen coated on the NC membrane can effectively improve the specificity of the detection. Meanwhile, the use of mAbs can overcome the bias between batches of pAbs, thus it is suitable for standardized production of antigen test strips (Fig. 2b). Li et al. had established an immunochromatographic strip for the detection of the spike protein of SARS-CoV-2 [22]. This strip can detect SARS-CoV-2 spike protein in subunit vaccine with a detection limit of 62.5 ng/mL within 15–30 min (Fig. 3b). This strip monitors vaccine quality by detecting the antigen content of spike protein, providing technical support for the early diagnosis of the outbreak of COVID-19.

Li et al. [23] had developed an immunochromatographic strip based on two mAbs against HA protein for rapid detection of H7 subtype avain influenza viruses. The detection limit of the strip was 2.4 log10EID50/0.1 mL for chicken swab samples, which provided an effective approach for the rapid and early detection of H7 subtype avain influenza viruses (Fig. 3c). Liu et al. [24] has developed a strip for the optical determination of influenza virus H3 subtype. The strip utilized gold nanoparticles (AuNP)-coated polystyrene latex microspheres (PS) based on two mAbs against HA protein of H3. It showed a detection limit of 0.016 hemagglutination unit, assisting early determination of influenza virus infection (Fig. 3d).

3.3. Combined mAb labeling-mAb capture format

For pathogenic microorganisms with large antigenic epitopes, the variation of epitopes may lead to evasion of pathogenic microorganisms by the above two detection formats. The combination mAbs that recognize different antigenic sites are labeled with colloidal gold. Its efficiency of epitope recognition greatly improves the sensitivity of the test (Fig. 2c).

4. LFIAs for antibodies detection

Indirect LFIA is used to capture antibodies such as IgG, IgM and IgA. IgM antibodies are commonly used as early detection of infection; IgG antibodies are for the monitoring of immune antibody titers or the detection of differential diagnosis marker antibodies; IgA antibodies are applied to evaluation of mucosal immunity. There are four formats of LFIAs for antibodies detection.

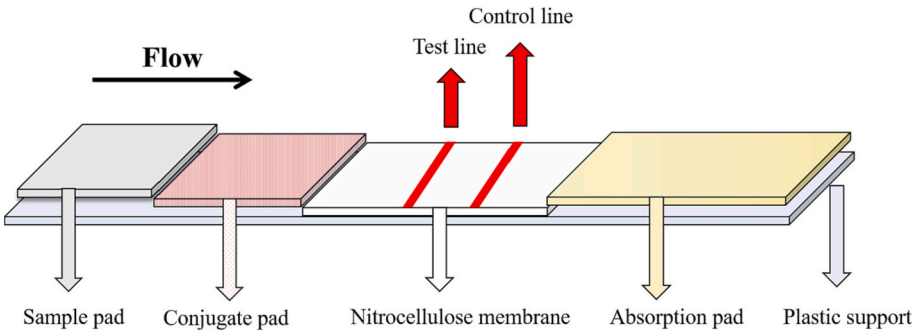

Fig. 1. Schematic diagram of a conventional LFIA structure.

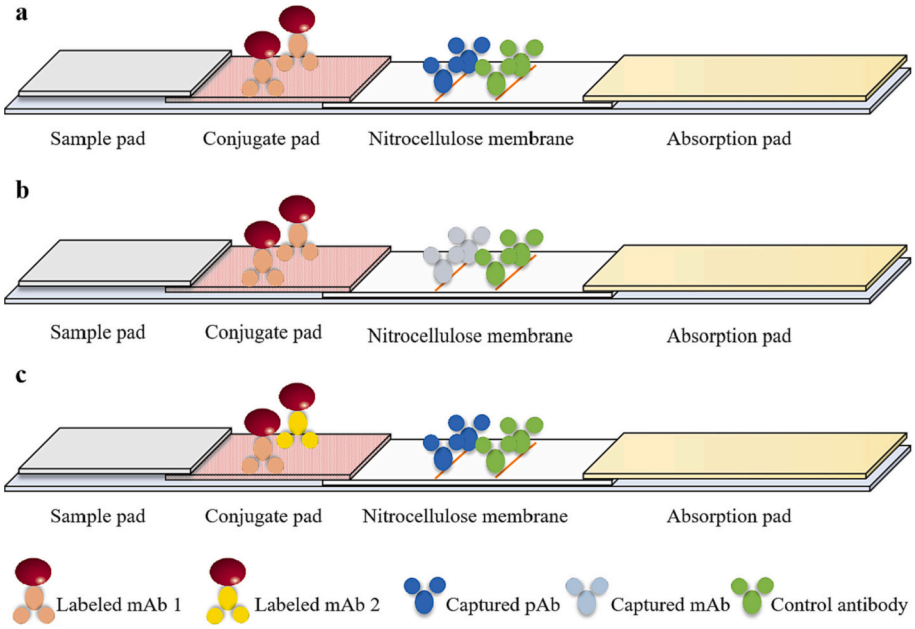

Fig. 2. Antigen detection format diagram of LFIAs. **a**: mAb labeling-pAb capture format; **b**: mAb labeling-mAb capture format; **c**: combined mAb labeling-mAb capure format.

4.1. Secondary antibody labeling-antigen capture format

The conjugate pad is prepared by labeling anti-species IgG antibody or bacterial immunoglobulin binding protein A/G with nanomaterial. The specific antigen works as a capture antigen and protein A/G or IgG antibody works as a control antibody, which are coated on the NC membrane as T line and C line, respectively (Fig. 4a). The labeled antibody can bind to all immunoglobulins in blood or serum samples, non-specific antibodies with immunoglobulins significantly affect the generation of specific immunological complexes, thus affecting the results. In particular, low sensitivity to trace specific antibody often cause false negative results. A gold nanoparticle strip discriminating Foot-and-Mouth disease virus (FMDV) vaccinated animals from infected animals was established [25]. SPA conjugated with colloidal gold nanoparticles were used as a probe. Two proteins of FMDV were coated as test line 1 and test line 2 (T1 and T2) and the goat anti-pig antibody IgG was coated as a control line, as shown in Fig. 5a. The strip showed the specificity of T1 and T2 were 95.17 % and 100 %, which was in accordance with commercial ELISA kits. This strip enables monitoring titers of O-type FMDV antibody on site.

4.2. Antigen labeling-secondary antibody capture format

Nanomaterial labeled antigen as a probe can interact with analyte in the sample to form immunological complexes, which captured by anti-species IgG antibody or protein A/G on T line, and then react with analyte-specific antibody on C line (Fig. 4b). Antigens as probe reduce the interference of non-specific immunoglobulins and improve the sensitivity. Non-specific antibodies have chances to bind to the secondary antibody or protein A/G, which affects the generation efficiency of specific complexes. The affinity of the capture antibody to immunoglobulin is much higher than that of labeled antibody (about 10 times), thus reducing the non-specific bindings. Meanwhile, the diluted serum with less interference of non-specific antibody boost sensitivity. Varieties of strips aiming at detection of diseases, particularly swine diseases have been established. (Table 1 and Fig. 5).

4.3. Antigen sandwich format

Most of the antibodies are multivalent antibodies (IgG, IgE and serum IgA are bivalent, and IgM is tenvalent), which can form antigen-specific antibody-labeled antigen complexes at T line. Nanomaterial labeled one antigen as a probe can interact with analyte in the sample to form immunological complexes, which captured by the other antigen on T line, and then reacted with antigen-specific antibody on C line (Fig. 4c). The color intensity is positively correlated with the antibody level. The double-antigen sandwich format eliminates the interference of non-specific immunoglobulins on the formation of labeled antigen-antibody complexes. When the tested serum contains a small amount of target antibody, the excess labeled antigen may block the binding site of the specific antibody resulting in false negative results.

4.4. Antibody blocking format

Antibody blocking format enables detection of specific antigenic epitopes or differential diagnosis of labeled antibodies through high-affinity mAbs. The blocking test strip established on the base of neutralizing mAbs. It detects the neutralizing antibody and evaluates the titer of the neutralizing antibody. Also, the antibody blocking format can effectively exclude the interference of non-specific antibodies. Nanomaterial-labeled antigens or neutralizing antibodies are key components of this test. PAb or mAb plays a role as blocking antibody immobilized on T line, and anti-mouse IgG antibody is coated on C line (Fig. 4d). An optimal concentration of antigen is critical to antibody diluent. When the sample does not contain analyte-specific antibodies, the antigens in the diluent bind to labeled antibodies from the conjugate pad and then react with capture antibody on T line, and those of rest intercept on control line, representing a negative result. When the sample contains the analyte-specific antibodies, antigen-antibody binding occurs in the first step, and terminates the following reaction, only C line displays, indicating a positive result. The presence of specific antibodies can significantly inhibit or completely block the formation of antigen-labeled antibody complexes, which cannot be effectively captured by the detection antibodies at the test blot, leading to the T line significantly weakened or completely disappeared. The labeled antibody, however, can be capture by the control antibody at C line, which

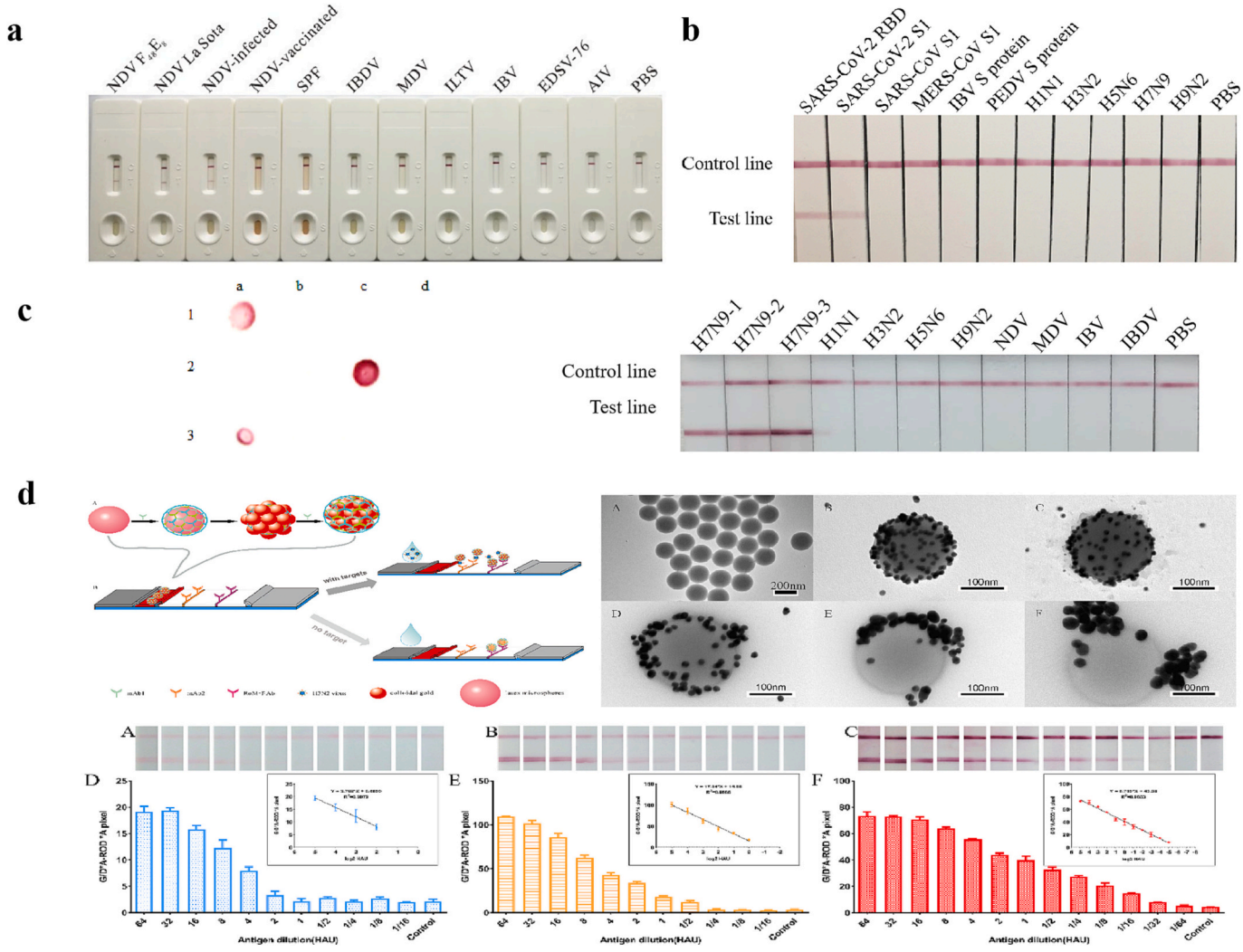

Fig. 3. LFIA for antigens detection developed in KLAI of HAAS. **a**: The LFIA for NDV antigen detection [21]; **b**: The LFIA for SARS-CoV-2 antigen detection [22]; **c**: The LFIA for H7 subtype avain influenza virus detection [23]; **d**: The LFIA for H3 subtype influenza virus detection with high sensitivity [24].

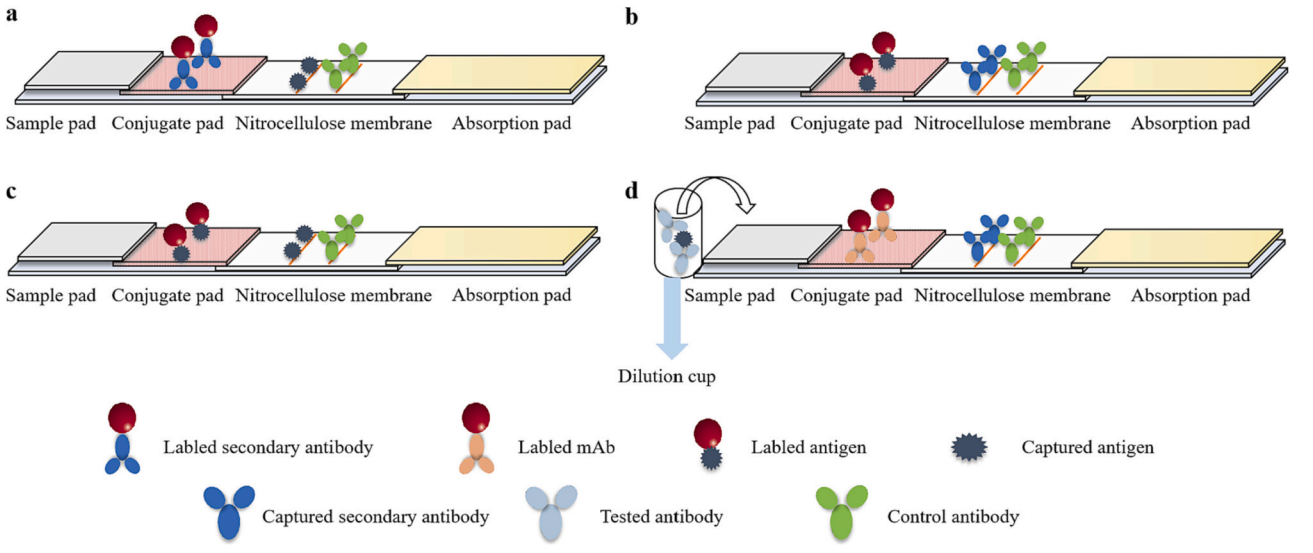

Fig. 4. Antibodies detection format diagram of LFIAs. **a**: Secondary antibody labeling-antigen capture format; **b**: Antigen labeling-secondary antibody capture format; **c**: Antigen sandwich format; **d**: Antibody blocking format.

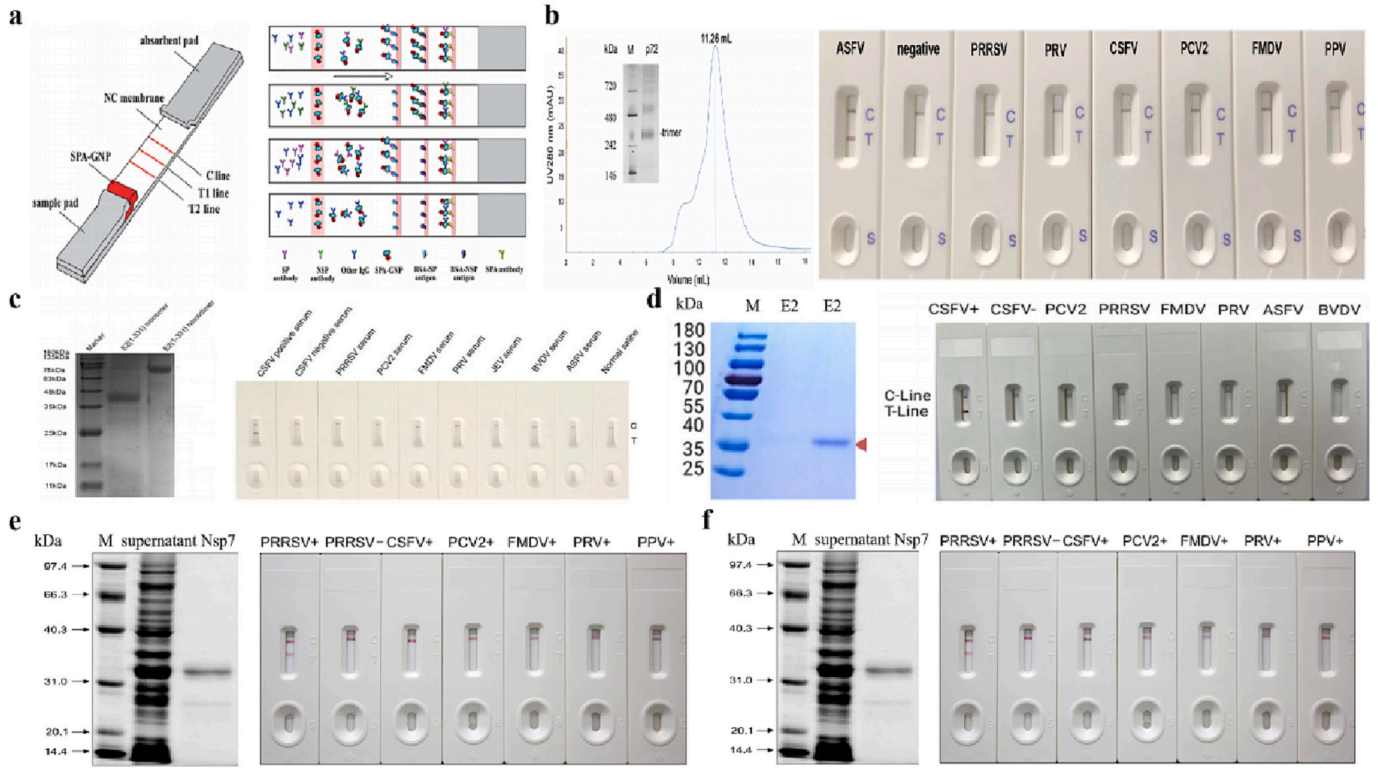

Fig. 5. LFIAs for antibodies detection. **a**: The LFIA for FMDV antibody detection; **b**: The LFIA for African swine fever virus (ASFV) antibody detection; **c** and **d**: LFIAs for Classical swine fever virus (CSFV) antibody detection; **e**: The LFIA for Porcine reproductive and respiratory syndrome virus (PRRSV) antibody detection; **f**: The LFIA for Pseudorabies virus (PRV) antibody detection.

Table 1
Antibody detection strips for different diseases.

Diseases	Antigens	Expression systems of antigens	Test line	Control line	Detection limit	References
African swine fever virus	P72 protein	HEK 293	SPA	Anti-p72 pAbs	1: 409,600	[26]
Classical swine fever virus	E2 protein	Bac-to-Bac baculovirus	SPA	Rabbit anti-E2 pAbs	1: 102,400	[27]
Classical swine fever virus	E2 protein	Transgenic rice endosperm	SPA	Anti IgG	1:128,000	[28]
Porcine reproductive and respiratory syndrome virus	Nsp7 protein	*Escherichia coli*	SPA	Anti-Nsp7 antibodies	1: 3200	[29]
Pseudorabies virus	GB protein	*Escherichia coli*	SPA	Swine anti-PRV antibody IgG	96 % compared with commercial ELISA	[30]

represents a positive result. The color intensity of the detection line is negatively correlated with the antibody level.

Ma [31] has developed an immunochromatographic strip for the detection of NDV antibody, which can distinguish vaccine-immunized animals from naturally infected animals within 10 min. The recombinant HN or F protein was adsorpted in an antigen pad or pre-incubation with serum. Then the anti-HN or anti-F mAb was labeled with colloidal gold as the detector. A chicken anti-NDV pAb and SPA were used as the T and C line, respectively. The colored C line without T line indicates a positive result, while both colored T and C lines indicates a negative result.

5. LFIAs for haptens detection

The haptens detection test strip mainly detects small molecule compounds of single antigenic epitope. The small molecule compounds are non-immunogenic or poorly immunogenic with small molecular weights. The hapten test strips are widely applied to the determination of antibiotics, pesticides, veterinary drugs, toxins, banned additives, drugs, hormones and heavy metals, etc. There are two formats of LFIAs for haptens detection.

5.1. Antibody labeling format

In the antibody labeling format, the conjugate pad is prepared by labeling the mAb that specifically recognizes the target antigen with nanomaterial. The target antigen coupled to a carrier protein (artificial antigen) is used as the capture antigen on T line, and the anti-species IgG antibody coated as C line (Fig. 6a). Sample without target leads to conjugated antibody captured by antigen on T line and then C line, indicating a negative result. Sample with target can be specifically recognized by conjugated antibody, thereby blocking the binding of the conjugated antibody to the capture antigen on T line. The presence of the target antigens interfere with blocking of antibody captured or completely blocked, significantly reducing the color appearing on T line. The unblocked labeled-antibody is recognized by C line, indicating a positive result. There were a series of test strips for the detection of haptens developed in KLAI of HAAS (Table 2).

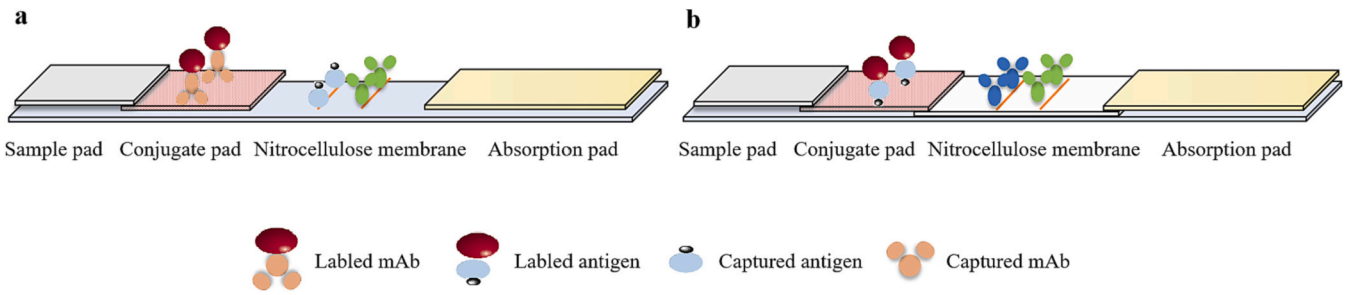

Fig. 6. Haptens detection formats diagram of LFIAs. **a**: Antibody labeling format; **b**: Antigen labeling format.

Table 2
Examples of haptens detection strips developed in the past five years.

Detection targets	Samples	IC_{50} values	Detection limits	Developed time	References
Xylazine	Milk	0.590 ng/mL	0.100 ng/mL	2022	[32]
Imidocarb	Milk	0.400 ng/mL	0.078 ng/mL	2021	[33]
Diminazene	Milk	5.200 ng/mL	1.200 ng/mL	2020	[34]
Bacitracin zinc	Milk	3.160 ng/mL	0.820 ng/mL	2020	[35]
Nitroxynil	Milk	5.716 ng/mL	1.146 ng/mL	2020	[36]
Danofloxacin	Milk	0.513 ng/mL	0.092 ng/mL	2019	[37]
Antibiotics	Milk	0.040 ng/mL	0.216 pg/mL	2018	[38]
Oseltamivir phosphate	Egg and chicken meat	2.56 and 2.63 μg/kg	0.43 and 0.42 μg/kg	2018	[39]

5.2. Antigen labeling format

In the antigen labeling format, the conjugate pad is prepared by labeling the target antigen (artificial antigen) with nanomaterial. The mAb specific for the target antigen is used as the capture antibody on T line, and the anti-carrier protein antibody immobilized as C line (Fig. 6b). Sample without target leads to conjugated antigen captured by antibody on T line and then C line, indicating a negative result. Sample with target can compete with the conjugated antigen to bind the detection antibody on T line making the antibody unable to be captured or completely blocked, reducing the color appearing on T line. The unblocked conjugated-antigen is captured by C line, which represents a positive result.

6. New trends of LFIAs

6.1. LFIAs for hyper sensitivity

With the continuous development of material technologies, more and more nanoparticles have been developed and applied to LFIAs to improve the sensitivity of conventional test strip. Nanomaterials have shown the great potential in the labeling of antigens and antibodies owing to the special structure level, strong adsorption capacity, good orientation performance, biocompatibility and structural compatibility [40]. Nanomaterials have been used in developing LFIA for various kind, including gold nanoparticles (GNPs), carbon nanoparticles (CNPs), colloidal selenium nanoparticles (SNPs), quantum dots (QDs), fluorescent microspheres and magnetic nanoparticles (MNPs). The advantages and disadvantages of different nanoparticles used in LFIA are shown in Table 3.

GNPs are in a stable colloidal state formed by electrostatic action and the polymerization of chloroauric acid under the reduction of trisodium citrate into gold particles of a specific size. GNP has a diameter of 1–150 nm and is purple color. Colloidal gold is widely used as LFIA-labeled probe on account of the characteristics of bright color, easy preparation, good biocompatibility, high stability, chemical traceability, and fine optical properties [57]. GNPs are a class of colored particle markers that can realize qualitative or semi-quantitative detection, with particle sizes ranging from 10 to 100 nm. The high stability, easy preparation, non-toxicity, easy conjugation and no activation characteristics of GNPs make them widely used in LFIAs [58]. Colloidal selenium is a nanoscale selenium particle with surface effect and small size effect obtained by adjusting the reaction conditions. It is widely used in LFIAs with characteristics of low cost, simple labeling with proteins, uneasy to coagulate after labeling, and easy adjustment of particle size range. Qds are spherical or quasi-spherical fluorescent semiconductor nanocrystals with a particle size ranging from 2 to 20 nm. QDs are considered to be the most potential biomarkers in LFIA, because of excellent fluorescence properties such as strong photochemical stability, long fluorescence lifetime, narrow and symmetrical emission spectrum [59]. Up conversion nanoparticles (UCPs) are fluorescent substances consisting of a host

Table 3
Advantages and disadvantages of different nanoparticles used in LFIAs.

Nanoparticles	Advantages	Disadvantages	References
Gold nanoparticles	Easy to prepare and functionalize, good biocompatibility, low cost, easy-to-read results, detectable with the naked eye	Low sensitivity, requires reader for quantification	[41–43]
Carbon nanoparticles	High signal-to-noise ratio, stable, functional, non-toxic	Qualitative or semi-quantitative, background interference	[44,45]
Colloidal selenium nanoparticles	Rust-colored, easy to prepare and handle	Difficult to discern the results with the naked eye	[46]
Quantum dots	High optical stability, wide absorption spectrum, narrow emission band, strong stability and high sensitivity	Easily quenched of fluorescence, toxic, high fluorescence background, require a fluorescence reader for quantification	[47–51]
Up conversion nanoparticles	Long fluorescence lifetime, easy functionalization, stable, tunable emission color, no background fluorescence interference, high sensitivity	require a fluorescence reader for quantification	[52–54]
Magnetic nanoparticles	Easy surface functionalization, magnetic properties, long signal duration and low background noise	Require a magnetic readeror magnetic field sensor	[55,56]

matrix, absorbers and emitters. UCPs are newly developed fluorescent probes that excited by low-energy near-infrared light and emits high-energy visible light. UCPs are ideal fluorescent nanomaterials with little damage to biological tissues and strong penetrating ability [60]. MNPs refer to nano-sized magnetic materials (represented by Fe_3O_4), which show satisfactory biocompatibility and magnetic orientation. MNPs are easy to prepare to couple with antibodies coated on immunomagnetic beads [61].

6.2. LFIAs for high-throughput detection

Typical LFIAs are suitable for point-of-care tests due to their portability, simplicity, cost-effectiveness, and rapid detection of target biomarkers [62]. However, detecting a single biomarker in typical LFIAs is not conducive to high-throughput diagnosis. Consequently, multi-biomarkers detection of LFIAs have been extensively studied in recent years [63]. Three typical structures are detection of multi-targets in a single-strip, a dual-strip with multi-targets, and microarray, integration of lateral flow with microanalysis. (Fig. 7) [64].

The popular strategy for the LFIA multiplexing is to design several T lines or dots on the immunochromatographic strip by using gold nanoparticles (GNPs), quantum dots, or colored/fluorescent microspheres as carriers. Fluorescent nanoparticles require additional equipment for excitation, thus colored nanoparticles visible to naked eyes have been more extensively researched [65–68]. Several individual strips can be combined into a special strip for samples to be collected only once and distributed to each strip in parallel flow. The advantages of this method rely on the large multiplexing capability and the absence of mutual interference between analytes that occur independently on a single strip [69]. Since a single test strip or a multi-test strip limited by quantitative capability and diagnostic validity, the combination of lateral flow and protein chip technology has been developed to achieve rapid and accurate detection of diagnosis of infectious diseases [70].

6.3. LFIAs for digital detection

Conventional LFIAs are convenient yet unable to achieve quantitative detection, and the bias in visual readings limit the application of LFIAs [71]. Thus, LFIAs with integrated optical readers to convert visual signals into more precise quantitative results is an acceptable approach. Instrument-based optical detection and electrochemical detection in conjunction with LFIAs are discussed.

Combining optical readers with LFIA make results to be easily obtained, especially in fields of genotyping and healthcare [72]. The imaging system accommodates a wider range of labeling systems with various color or optical properties, providing a more versatile instrument platform for LFIA. It is performed in conjunction with LFIAs by placing the test strip in a controlled lighting environment, where a complementary metal-oxide-semiconductor camera or high-density charge-coupled device captures images of the test and control lines [73]. Hundreds of millions of mobile phone users worldwide make mobile an attractive distributed platform for the future diagnostic market. The mobile phone camera based on CMOS imaging sensor can provide high image quality. Optical reader added up to LFIA will greatly improve its efficiency and convenience.

7. Outlooks

LFIAs based on three conventional systems of antigen, antibody and hapten have flourished since the establishment. They are extensively available in diagnosis, prognosis, screening, and monitoring of diseases in veterinary industry and human healthcare in hospital wards, clinics, health centers and self-diagnosis. IgM or IgG antibody test strips known to world since the outbreak of the COVID-19 in 2019. In order to monitor the SARS-CoV-2 and achieve effective control of the epidemic, antigen test strips based on antigen-antibody responses have been entrusted with detecting throat swabs or nasal swabs. However, a series of mutant viruses with stronger transmission of COVID have been derived, including the Alpha, Beta, Delta and Omicron variants.

The demands for quantitative LFIAs increase as the LFIA market expands [74]. New trends of novel labels, multiplex and digital assays are increasingly integrated with LFIAs with the rapid innovation of diagnostic technologies. Through the reader, nanoparticle tags captured in the test area are excited by external physical stimuli, such as lasers, electrical potentials or magnetic fields, resulting in amplified signals [75]. LFIAs are the backbone of rapid point-of-care diagnostics, with the potential to enable early case management and change in the epidemiology of infectious diseases. Emergent requirements from users for multiplexed systems capable of detecting multiple biomarkers simultaneously due to the complexity, multiple symptoms, and multiple infectious states of human disease. Imaging systems are also used for quantitative analysis of LFIAs. Cho I [76] used confocal Raman imaging combined with silver-enhanced technology to detect influenza B virus with sensitivity 1000 times higher than that of ordinary method. Mobile telephony (cell phone) healthcare is an emerging field as a next-generation point-of-care diagnostic platform.

8. Conclusions

The unique and remarkable properties of LFIAs have contributed to the detection of disease biomarkers and infectious agents in the fields of drugs, food, agriculture and environmental safety through three major detection systems. The principles of LFIAs have remained for decades, while the updates continue to occur. The sensitivity and reproducibility of LFIAs are promoted through novel labels and digitized devices, multiplex. Moreover, LFIAs effectively performed outside the laboratory with continued refinement. They offer the point-of-care diagnostics for developing countries both on site and in clinical settings.

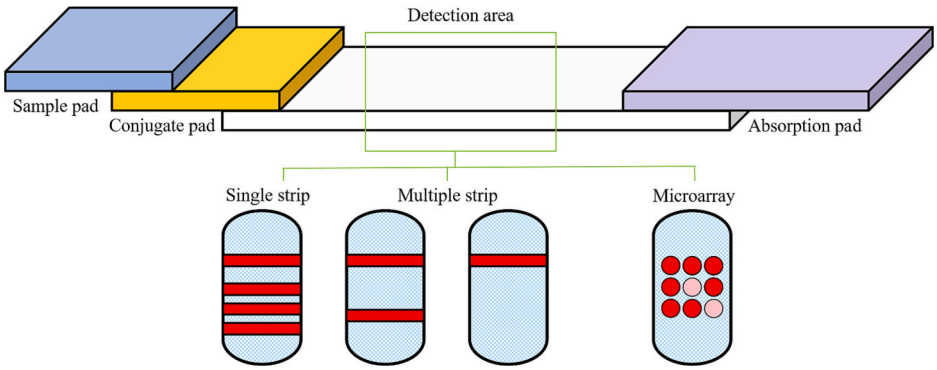

Fig. 7. Schematic of three multiplex LFIAs.

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Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Ge Li: Writing – original draft. **Qingmei Li:** Supervision. **Xun Wang:** Data curation. **Xiao Liu:** Visualization, Investigation. **Yuhang Zhang:** Writing – review & editing. **Rui Li:** Supervision. **Junqing Guo:** Conceptualization, Methodology, Software. **Gaiping Zhang:** Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Datasets that support the current study are available from the corresponding author, [RS], upon reasonable request.

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