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Lateral flow assays (LFA) as an alternative medical diagnosis method for detection of virus species: The intertwine of nanotechnology with sensing strategies

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ABSTRACT

Viruses are responsible for multiple infections in humans that impose huge health burdens on individuals and populations worldwide. Therefore, numerous diagnostic methods and strategies have been developed for prevention, management, and decreasing the burden of viral diseases, each having its advantages and limitations. Viral infections are commonly detected using serological and nucleic acid-based methods. However, these conventional and clinical approaches have some limitations that can be resolved by implementing other detector devices. Therefore, the search for sensitive, selective, portable, and costless approaches as efficient alternative clinical methods for point of care testing (POCT) analysis has gained much attention in recent years. POCT is one of the ultimate goals in virus detection, and thus, the tests need to be rapid, specific, sensitive, accessible, and user-friendly. In this review, after a brief overview of viruses and their characteristics, the conventional viral detection methods, the clinical approaches, and their advantages and shortcomings are firstly explained. Then, LFA systems working principles, benefits, classification are discussed. Furthermore, the studies regarding designing and employing LFAs in diagnosing different types of viruses, especially SARS-CoV-2 as a main concern worldwide and innovations in the LFAs' approaches and designs, are comprehensively discussed here. Furthermore, several strategies addressed in some studies for overcoming LFA limitations like low sensitivity are reviewed. Numerous techniques are adopted to increase sensitivity and perform quantitative detection. Employing several visualization methods, using different labeling reporters, integrating LFAs with other detection methods to benefit from both LFA and the integrated detection device advantages, and designing unique membranes to increase reagent reactivity, are some of the approaches that are highlighted.

1. Introduction

Viruses are infectious entities consisting of one kind of nucleic acid, either DNA or RNA, encapsulated by a protein shell, which their diameters vary from 16 nm to over 300 nm. They sometimes are wrapped in a lipid membrane in some species. Since viruses do not contain ribosomes or any other cell-like organelles, their only means of reproduction are infecting living cells and replicating using host cell ribosomes. Their nucleic acid consists of genes that

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List of abbreviations

acpcPNA	Pyrrolidinyl peptide nucleic acid
AIDS	Acquired immunodeficiency syndrome
AIV	Avian Influenza virus
anti-FITC	Anti-fluorescein isothiocyanate
Au NP-DPs	Au NP-detector probes
Au NPs	Gold nanoparticles
Au/Pt	Gold-platinum
AUDG	Antarctic thermolabile uracil-DNA-glycosylase
BART	Bioluminescent assay in real-time
BKV	BK virus
BPE	1,2-Bis(4-pyridyl)ethylene
BR	Binding ratio
BRPA	Betaine-assisted recombinase polymerase assay
BSA	Bovine serum albumin
CAST	Chinese academy of science test
cDNA	Complementary DNA
CDs	Carbon dots
CDSNs	Carbon dots/SiO2 nanospheres
CHA	Catalytic hairpin assembly
CHIKV	Chikungunya virus
CL	Control line
CLIA	Chemiluminescence assay
CMV	Cytomegalovirus
COPs	Control probes
CPs	Capture probes
CRISPR	Clustered regularly interspaced short palindromic repeats
CuO NPs	Copper oxide nanoparticles
DENV	Dengue virus
DIG	Digoxigenin
DPs	Detector probes
DRELFA	Dual recognition element LFA
dsDNA	Double-stranded DNA
EBV	Epstein-Barr virus
EBVNA-1	EBV nuclear Ag-1
eLFA	Electrochemical LFA
ELFI	Electrochemical lateral flow immunosensor
ELISA	Enzyme-linked immunosorbent assay
FCS	Fluorescent CD-based silica
FITC	Fluorescein isothiocyanate
FNDPs	Fluorescent nano-diamond particles
FRET	Fluorescence resonance energy transfer
GAC	Goat anti-chicken
GAH	Goat anti-human
GAM	Goat anti-mouse
GAR	Goat anti-rabbit
GNSs	Gold nanostars
GO	Graphene-oxide
GPs	Glycoproteins
HADV	Human adenovirus
HAV	Hepatitis A virus
HBB	Human β-globin
HBeAg	HB e-antigen
HBsAb	Hepatitis B surface antibody
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDTMS	Hexadecyltrimethoxysilane
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HRP	Horseradish peroxidase
HSV-2	Herpes simplex virus type 2
IAV	Influenza A virus
ICC	Immunocytochemistry
IF	Immunofluorescence
Ig	Immunoglobulin
IHC	Immunohistochemistry
IR	Infrared
IVB	Influenza B
LAMP	Loop-mediated isothermal amplification
LDP NPs	Lanthanide-doped polystyrene NPs
LFAs	Lateral flow assays
LFD	Lateral flow dipstick
LFIA	Lateral flow immunoassay
LNSM	LFA-NanoSuit method
LOC	Lab-on-chip
LOD	Limit of detection
mAb	Monoclonal antibody
MAC	IgM Ab capture
MAH	Mouse anti-human
MB	Methylene blue
MBA	4-mercaptobenzoic acid
MBPs	Multibranched peptides
MGITC	Malachite green isothiocyanate
MNPs	Magnetic NPs
MPXV	Monkeypox virus
MQDNBs	Magnetic quantum dot nanobeads
NAA	Nucleic acid amplification
NASBA	Nucleic acid sequence-based amplification
NBA	Nile blue A
NC	Nitrocellulose
NIR	Near-infrared
NPs	Nanoparticles
NSB	Non-specific binding
NSP1	Nonstructural protein 1
OHT	Oseltamivir hexylthiol
OPS	Onset of post-symptoms
OPV	Orthopox virus
ORF1ab	open reading frame
pAbs	Polyclonal antibodies
PCL	Polycaprolactone
PCR	Polymerase chain reaction
PDMS	Polydimethylsiloxane
PE	Polyethylene
pGAM	Polyclonal goat anti-mouse
POCT	Point of care testing
PSR	Polymerase spiral reaction
PSR	polymerase spiral reaction
QDs	quantum dots
RAM	Rabbit anti-mouse
r-HIV-1env	Envelope glycoproteins of HIV-1
r-HIV-2env	Envelope glycoproteins of HIV-2
RPA	Recombinase polymerase amplification
RT-LAMP	Reverse transcription-LAMP
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SAS	Signal amplification system
SB	Specific binding
SELEX	Systematic evolution of ligand by exponential enrichment
SEM	Scanning electron microscope
SERS	Surface-enhanced Raman scattering
SFTSV	Severe fever and thrombocytopenia syndrome virus
SPGE	Screen-printed gold electrode

ssDNA	Single stranded DNA
STV	Streptavidin
TBEV	Tick-borne encephalitis virus
TCA	Thermal contrast amplification
TL	Test line
TP	Treponema pallidum
UC NP-LFA	Up-conversion NP-based LFA
UC NPs	Upconversion NPs
UPT-LFA	Up-converting phosphor technology-based LFA
VACV	Vaccinia virus
VLPs	Virus-like particles
WB	Western blot
WNV	West Nile virus
ZKV	Zika virus

code structural components, regulatory proteins, and enzymes [1,2]. Viruses evolve through mutations which double-stranded (dsDNA) viruses' mutation rate is as high as 0−7 per nucleotide per year. This rate is higher than in RNA viruses on the order of 3−10 per nucleotide per year [2]. The mutagenic nature of viruses sparked incidents like the sudden emergence of the most recent Covid-19 and gave rise to variants of avian influenza that can be transmitted to humans [3]. Viruses can be also transmitted via aerosol and airborne droplets, fecal-oral, insect transmission, sexual transmission, blood-borne, and other unknown ways and can be entered human body through respiratory, gastrointestinal, and genital tracts, as well as skin, eye, placenta, transplants, and blood transfusion and they can exit the body through entering ways [4]. Currently, 224 virus species are known that can infect humans, every year, three to four new species are discovered, and there is a lot more to be found [5,6]. Occurrences of emerging and re-emerging viral infections and deadly viral diseases have significantly affected human health. They can cause respiratory, intestinal, skin, single organ (e.g., liver, lung, heart, etc.), multi-organ, neurologic, hematologic, immunosuppressive diseases [7]. Furthermore, viruses can alter host cell's genetic material and cause benign and malignant tumors [4]. Due to the urgency and severity of viral infections, accurate, reliable, user-friendly, available, rapid, and low-cost detection methods are necessary [8]. Established approaches such as virus isolation methods like virus culture, nucleic acid-based detection methods like Polymerase chain reaction (PCR), and serologic methods like enzyme-linked immunosorbent assay (ELISA) are widely used in clinical settings. Although these methods are highly sensitive and specific, they require expensive equipment and expert operators and lack the rapidity of newer detection methods [9]. Biosensors are one the alternative methods to conventional methods. A biosensor is a diagnostic device consisting of an electrical transducer that transforms biological responses from a recognition receptor to an electrical signal detected, correlated, and demonstrated by an electrical detector [10,11]. Biosensors can be classified by numerous approaches, considering their electrical transducer, their bio element, and the detected component as the categorization criterion [12,13]. Biosensors, however, have some limitations (e.g., low sensitivity) that can be overcome using nanotechnology [14−16].

Lateral flow assays (LFA) are the most recently used approaches for biosensing and quantifying numerous analytes, including different viruses [17]. The technical principle of LFAs was derived from the latex agglutination test. The first enzyme immunoassay was introduced in the 1960s, with several advantages like rapidity, ease of use, and long shelf life. The LFA principle was first popularized by the human pregnancy test that detected human chorionic gonadotropin in pregnant women urines, and since then, LFAs have been widely used in medicine and other areas like veterinary, agriculture, biowarfare, food, environmental health and safety [18]. LFAs application in medicine are highly versatile and can be used for diagnosis, prognosis, screening, monitoring, and surveillance. Unlike conventional methods, they can be used outside of high-tech laboratories and are applied in hospital wards, clinics, health centers, physicians' offices, and also can be used by patients themselves for self-diagnosis [17]. This review aims to discuss the recent developments of LFA devices in detection of different viral diseases. Various characteristics of LFA detection approaches used for determination of various kinds of viruses are comprehensively discussed. Additionally, current challenges and problems as well as different strategies considered in the recently introduced LFA-based portable systems for eliminating shortcomings and enhancing their efficiency for the effective determination of different kinds of viral species are highlighted in this review.

2. Clinical and conventional approaches for the recognition of viruses

Since their discovery, scientists have developed multiple methods for virus detection. These methods can be classified into various clinical approaches including serological methods, hybridization methods, methods based on PCR and isothermal amplification methods. Although there are a great variety of detection tests, only some are suitable for clinical use. Here, brief discussions mainly focused on the serological and nucleic acid-based approaches have been provided in the following.

2.1. Serological methods

Serological methods are clinical procedures characterized by detecting immunoglobulins in various samples and consist of multiple tests like immunohistochemistry (IHC), agglutination test, flow cytometry, radioimmunoassay, and ELISA [19,20]. Despite being one of the oldest detection techniques, serological methods are still widely used in clinical recognitions owning to their high sensitivity, specificity, ease of use and cheapness. Based on the manifestation of anti-viral immunoglobulin (Ig) using virus antigen (Ag) in the sample, serological approaches can be employed to diagnose and monitor viral diseases [21]. ELISA is one of the serological methods that use enzyme-labeled antibodies (Abs) to detect viral Ags, making it feasible for detecting Abs in large sample groups [22]. The ELISA method is initiated by coating the plate with specific antigens or antibodies that detect the desired analyte, and then the plate is then washed to remove any unbound substrates. Then animal proteins like ovalbumin, aprotinin, etc., are used to block unbound spots in the container. After another washing step, an enzyme-linked detection antibody is added that binds to the primary antibody. Another washing step is performed, then a substrate/chromophore is added, producing a color detected and measured by the reader to quantify the detected analyte [23]. ELISA method is sensitive, quantitative, and relatively low-cost for significant sample detection. With the emergence of the Zika virus (ZKV) in 2015, several ELISAs were manufactured to detect this virus, and several studies evaluated these methods. In a study, for assessing molecular and serological procedures caused by ZKV infection, the authors used Dengue virus (DENV), ZIKV IgM ELISAs to detect and evaluate these techniques in pre-diagnosed patients by examining IgM cross-reaction among tick-borne encephalitis virus (TBEV), DENV and ZKV. In this study, a specificity around 97.4% was reported for nonstructural protein 1 (NSP1) Ag-based

ZKV IgM ELISA (Euroimmun) [24]. In another study, to compare three ZKV IgM screening ELISAs, samples from pre-diagnosed patients in a Colombian hospital were tested with each of these methods. Two of the evaluated ELISAs, the CDC IgM Ab capture (MAC)-ELISA and the In Bios MAC-ELISA, had an agreement above 90% in their overall results; the third evaluated ELISA, Euroimmun ZKV IgM ELISA performance differed from the two others, with an overall agreement of 50% compared to these two ELISAs. However, it wasn't determined whether Euroimmun low agreement with the other two approaches has resulted from its higher specificity to ZKV Ags or it had a lower sensitivity to the tested analyte [25]. In another study, the Euroimmun ZKV IgM ELISA sensitivity was reported to be 29.8% [26]. Agglutination test, immunocytochemistry (ICC), immunohistochemistry (IHC) and immunofluorescence (IF) are considered to be other serological methods based on Abs that are rapid, easy to perform, highly specific and sensitive.

2.2. Nucleic acid-based detection approaches

Polymerase Chain Reaction (PCR) and Reverse transcription PCR (RT-PCR) are the most important nucleic acid-based detection methods and are recognized to be a gold standard for detecting viruses due to their high sensitivity and specificity. On the other hand, real-time PCR is another powerful detection method that is based on the amplification of virus sequences. Continues measurement of fluorescent signals during the amplification reactions allows the detection instrument to quantify the target sequence. This method is not limited by the analyte concentration or other variables (e.g., cycling condition), which results in this method's high sensitivity. Due to these benefits, real-time PCR is considered the gold standard in virus detection [27]. In a study to evaluate two RT-PCRs for diagnosing ZKV, virus RNA was prepared by the Qiagen QIAamp viral RNA mini kit, and DENV and CHIKV were used as the negative control virus pathogens. RT-PCRs performed well and had an overall 96.1% sensitivity and 100% specificity, and showed an excellent agreement (Cohen's kappa = 0.93) [28]. In another investigation, the performance of the triplex RT-PCR was evaluated for the ZKV and it was found that RT-PCR has a sensitivity of 85% for serum components from diagnosed patients. However, the sensitivity is much lower in urine samples. This drop-in sensitivity can be explained by sample collection and processing effect on RT-PCR's sensitivity [29]. Furthermore, a TaqMan MGB real-time PCR assay was developed to detect the Influenza *H5N8* subtype. For this approach, two sets of primers were designed for H5 and N8 sequences, and samples were gathered from mice infected with the H5N5 Influenza virus for specificity and sensitivity evaluation. The results were consistent with Influenza A virus (IAV) real-time PCR and indicated that this method is sensitive and specific enough to be used for H5N8 detection [30].

Another nucleic acid-based detection approach is loop-mediated isothermal amplification (LAMP) which is highly specific and sensitive, easy to use, fast, and could be performed within 1 h. LAMP is another nucleic acid-based detection method and unlike PCR, it uses heat to denature dsDNA. LAMP employs four sets of primers to detect six distinct sequences on the target in one-step amplification in an isothermal state to generate 109 copies of DNA, thus increasing its specificity [31]. LAMP's low detection time and cost, high specificity and sensitivity, and being less prone to inhibitors make it an excellent detection tool in clinical settings. It is currently used in some studies for virus recognition. In a study, Gonzalez and coworkers developed a quantitative colorimetric LAMP to detect and amplify SARS-COV-2. LAMP test was coupled with pH indicator (phenol red), and the gene sequences that encoded the nucleocapsid proteins were targeted for detection and amplification. This colorimetric LAMP method performance was as good as reverse transcription quantitative PCR (RT-qPCR), with a sensitivity of 92.85% and specificity of 81.25%. In contrast to RT-qPCR, this approach was more rapid and required small amounts of RNA extract [32]. Moreover, a lab-on-chip (LOC) LAMP-bioluminescent assay in real-time (BART) was designed for detecting viral DNA. A LAMP was coupled with BART to detect and quantify the B19 virus. B19 virus causes fifth disease that is transmitted via respiratory tracts and causes mild rashes in children. The developed LOC was then verified through performance tests. The results were satisfactory for a POCT device and had a significant agreement with the conventional methods [33]. Another investigation describes a multiplex reverse transcription-LAMP (RT-LAMP) which was designed to simultaneously detect human Influenza viruses, coupling RT-LAMP with a colorimetric visualization system to determine results without requiring any extra steps. Multiple primers were designed for various subtypes of Influenza virus, for instance, H1N1, H3N2, H5N1, H5N6, H5N8, and H7N9. This device reached a sensitivity of 92.3% and 98.9% agreement with qRT-PCR [34].

3. Lateral flow assays (LFAs)

Although all of the above-mentioned detection methods have numerous advantages, they, however, have some serious limitations. Unlike other methods that directly detect viral components (Like nucleic acids or nucleoproteins), Ab-based serological methods are not suitable for early detection, and Ab levels are only detectable after infection. Although ELISA is a great tool for clinical settings like hospitals that routinely detect samples in large scales, it's not cost-effective for detecting small numbers of samples. RT-PCR requires high-skilled operators to perform, and is more expensive than other conventional detection methods. In addition, the RT-PCR procedure is very time-consuming, which makes it unsuitable for quick detection in clinical detection. LAMPs have retractions in their primer design and are susceptible to contamination [20,35,36]. Due to such limitations in the current approaches, a rapid, specific, sensitive, user-friendly, and affordable diagnostic method for virus detection is required.

The LFA-based devices may be a good substitute for POCT application in virus detection. LFA is a paper-based biosensor device that can rapidly detect various analytes (e.g., proteins, haptens, nucleic acids, and amplicons) from multiple types of samples (e.g., urine, saliva, sweat, serum, plasma, nasopharyngeal swabs, and stool) within 5–30 min [37–39]. The use of capillary forces in the nitrocellulose (NC) membrane speeds up and facilitates the efficiency of the assay. Furthermore, membrane fiber arrangements can filter out any unwanted analyte in the sample [40]. These tests can be applied in the clinical setting due to their user-friendliness and cheap manufacturing cost and are helpful for onsite decision-making [41]. In addition to these, LFAs have are stable in various environmental conditions and have a long shelf-life, require small sample volume, do not require pre-preparation for fluid samples or washing steps. LFAs mostly require little to no energy, but can be integrated with electronics, have a high potential for commercialization, and be used in various approaches [42]. For these benefits, LFAs are suitable devices for detecting viruses in any sample for clinical practice. LFAs have attracted considerable attention in recent years due to being rapid, cost-effective, user-friendly, and portable [43]. Due to their portability, LFAs can be used in deprived areas, and due to their user-friendliness, they can be performed without expert personnel [38]. LFAs can be classified into lateral flow immunoassay (LFIA), which uses antibodies as recognition elements, and nucleic acid lateral immunoassay (NAFLA), which detects amplicons produced during other nucleic-based amplification devices like PCR. LFIA [38]. Based on their detection

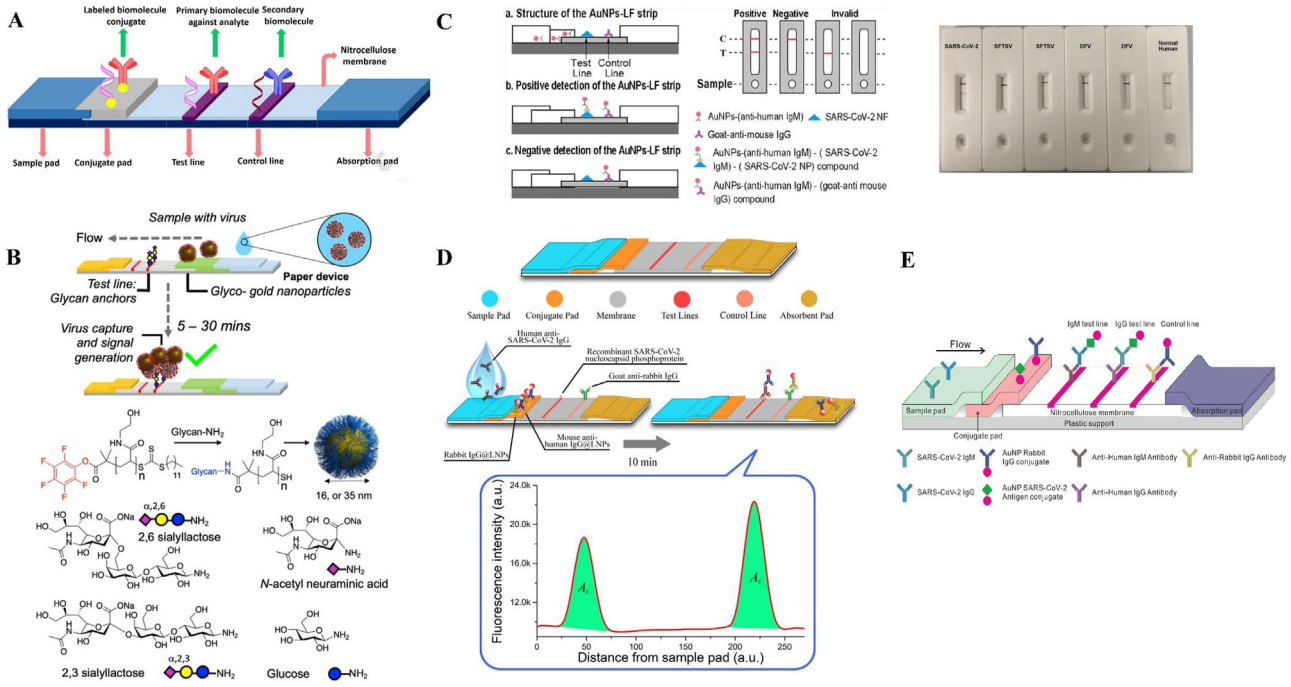

Fig. 1. A conventional LFA structure (A). Adapted by permission from Ref. [88]. A design concept for LFA, and the synthesis procedure of Au NPs (B). Adapted by permission form Ref. [45]. The Au NPs LF strip's operating principle and specificity of AuNP-LF strips for SARCS-CoV-2 (C). Adapted by permission from Ref. [46] Structure of a LNP-based LFA applied for SARCS-CoV-2 detection (D). Adapted by permission from Ref. [79]. Schematic depiction of a LFA with Au NPs coated by spike protein from *SARS-CoV-2* on the wicking membrane (E). Adapted by permission from Ref. [85].

approaches, the LFIAs can further be classified into sandwich type and competitive type [44]. As shown in Fig. 1A, a conventional sandwich LFA strip typically includes some pads such as a sample application pad, conjugate pad as well as NC membrane, and an absorbent pad. Upon introducing sample to the application pad, it will move to the conjugate pad. The conjugate pad contains labeling reporters conjugated to detector probes (which can detect the analyte) to form the reporter probes. The sample is added to the conjugate pad, and the fluid flows toward the NC membrane, which usually contains a test line (TL) and a control line (CL). The sample analyte binds to the detector probe in the conjugate pad or flows through the NC membrane. The TL includes the capture probes (CPs) which can detect the analyte in the immunocomplex. The CL comprises the control probes (COPs) that detect the detector probes. If the TL is detected, then the sample contains the analyte, and the CL should always be detected whether or not the sample contains the analyte. The test is not valid if the CL is not detected. The absorbent pad keeps the flow in the LFA, and the fluid is streamed towards it [42]. In the sandwich format, the analyte binds to both detector probe and capture probe, therefore it is suitable for large molecules. The competitive format is more suitable for small molecule like haptens. Competitive format itself can be divided into two setups. In the first setup, both analyte and the molecule that is conjugated to labeling reporter have a high affinity to bind with Abs on the TL, therefore the analyte compete with the reporter probe. In the second setup, the analyte attaches to the reporter probe, therefore blocks it from attaching to the Abs on the TL. In both formats, with higher concentration of analyte in the sample, the signal intensity on the TL decreases [44].

4. Viral infections determination using LFA sensing platforms

Table 1 summarizes various LFA-based sensing platforms developed in recent years for the determination of various viral infections. Additionally, the main properties of each LFA-based device are provided in this table indicating the interest in clinical assays for sensing different types of viruses using LFA biosensors. In this way, the recent developments regrinding each types of virus are comprehensively highlighted in the following subsections. Thus, various LFAs for detection of different viral infections are comprehensively reviewed taking a deeper look at their design, novelty, performance and analytical figures of merits.

4.1. Sars-Cov-2

In late 2019, Sars-Cov-2 reported from Wuhan city in China has led to a pandemic that is transmitted from person to person and can be presented as mild, moderate, to severe and fulminant disease. Since the discovery, multiple diagnostic methods using various samples (blood, stool, oral swabs, sputum, etc.) have been developed [72–74]. Numerous diagnostic tests like RT-PCR, clustered regularly interspaced short palindromic repeats (CRISPR) are nucleic acid-based, targeting multiple genes. Serological tests are also popular as ELISA, chemiluminescence assay (CLIA), western blot (WB), etc., are commonly used in clinical settings and are the first lines tests at screening these diseases. LFA is a novel method in diagnosing Sars-CoV-2 [75]. In a study, Hoste and coworkers evaluated two serological detection methods, ELISA and LFA, in detecting *anti*-Sars-CoV-2 nucleocapsid protein antibody. 1065 samples collected from Sars-Cov-2 patients and patients infected with other respiratory viruses were tested on both diagnostic devices. In this study, recombinant N protein was used at the TL, and a monoclonal antibody against BSA was used at the Cl. Black latex beads were conjugated with N protein and BSA to act as reporter probe. Results for LFA and ELISA showed acceptable sensitivity (91.2% and 100%) and specificity (100% and 98.2%), respectively [76].

Catalytic hairpin assembly (CHA) as an application reaction was utilized by Zou and coworkers for designing an isothermal and

Table 1
LFA-based sensing assays regarding the determination of different virus species.

Detected Virus	Detected analyte	Reporter probe	Capture probe	Control Probe	Sensitivity	LOD	Ref.
SARS-CoV-2	Spike protein	Au NPs	SARS-COV-2, S1 spike protein	2,3′-sialyl lactose-BSA	–	5 μg	[45]
SARS-CoV-2	Anti-SARS-CoV-2 IgM	Au NPs	SARS-CoV-2 NP	Anti-mouse IgG	100%	–	[46]
SARS-CoV-2	RNA	Au NPs	Anti-FITC Ab	Biotin	100%	–	[47]
SARS-CoV-2	Nucleoprotein Ag	Cellulose nanobead	Nucleoprotein Ag	Anti-human IgG antibodies	–	2 ng	[48]
SARS CoV-2	Anti-SARS-CoV-2 IgM-IgG - IgM	Au-NPs	Protein A & nucleocapsid proteins	Avidin	94.6%	–	[49]
SARS CoV-2	Anti-SARS-CoV-2 IgA	Au-NPs	Nucleocapsid antigen	staphylococcal protein A	–	–	[50]
SARS CoV-2	Anti-SARS-CoV-2 IgG	Au-NPs	SARS CoV-2	Anti-mouse IgG	69.1%	–	[51]
SARS CoV-2	Nucleoprotein and spike protein	Au-NPs	Mouse anti-human IgG and anti-human IgM	mouse *anti*-tylosin antibody	96%	–	[52]
IAV	Nucleoprotein Ag	Au/Pt NPs conjugated with anti-Influenza A NP	Anti-Influenza A Ab	Anti-mouse IgG	94%	–	[53]
IAV	Nucleoproteins	Ca-coated UC NPs conjugated with *anti*-AIV NP Ab	Anti-AIV NP Ab	GAM IgG	–	$10^{3.5}$ EID_{50}/mL	[54]
Tamiflu-resistant Influenza virus	Antiviral neuraminidase	Au NPs conjugated with OHT	Anti-AIV nucleoproteins (NP)	Tamiflu resistant NPs	–	5×10^2 PFU	[55]
IAV - IBV	IAV and IBV antigens	Europium	Monoclonal anti-influenza A and monoclonal anti-influenza B mouse IgG	polyclonal mouse IgG	68.9%	–	[56]
HIV, HCV, HAV	Antiviral Abs	Au NPs conjugated with protein A, BSA and proteinticles	Specific proteinticles	GAH Abs	100%	–	[57]
HIV	HIV-1 DNA	Au NPs conjugated with Raman reporter MGITC and DNA detection	Capture DNAs	Control DNAs	–	0.24 pg/mL	[58]
HIV	HIV DNA	QDs conjugated with hairpin DNA	Test-DNA	Control-DNA	–	0.76 pM	[59]
HIV, HCV, HBV	HIV, HCV, HBV Abs	QD nanobeads	HIV-1/2, HCV, HBV, and HBs antigens	GAM IgG	88.33–93.42%	–	[60]
HBV	HBV DNA	detProbe-Au-ssDNA	T-DNA	C-DNA	–	10^2 IU/mL	[61]
HBV	HBs Ag	UC NPs	AntiHBsAg mAb	RAM IgG	95.4%,	–	[62]
DENV	Anti-Dengue Ab	DYlight-800	Recombinant Dengue type 1 envelope	GAH IgG	95%	–	[63]
DENV	Dengue NS1	Magnetic beads	mAbs	GAM Ab	–	0.1 ng/ml	[64]
DENV	Dengue-1 RNA	Au NPs	dDNA	cDNA	–	1.2×10^4 pfu/mL	[65]
HPV	HPV16 E7 and HPV18 L1	SA-DNPs	Anti-FITC and anti-DIG	Biotin-BSA	–	5×10^5 copies	[66]
TORCH pathogens	IgM Abs	Au/hemin@MOF (metal organic framework)	Torch Ab	GAM IgG Ab	100%	–	[67]
EV	EV glycoprotein	RNs@Au	Anti-Ebola glycoprotein Ab	Anti-human Ab	–	2 ng/mL	[68]
EV	EV glycoprotein	AuNP	EE8 Ab	Anti-mouse immunoglobulin	85.7%	2.2 x 10^4 genome copies/ml	[69]
EV	EV glycoprotein	AuNP	mAb 1HK7 - mAb 2HK1	Goat anti-mouse Ig		0.23–0.37 ng/mL	[70]
SFTSV	SFTSV NP	Au NPs	mAbs	GAM IgG	–	1 ng/mL	[71]

nonenzymatic signal amplification system coupled with a lateral flow immunoassay (LFIA) strip-based device for signal amplification and sensitive RNA detection of SARS-CoV-2 that takes 90 min for completing diagnostic process. In this study, the hairpins DNA probes H1 and H2 were manufactured and were conjugated with biotin and digoxigenin (DIG). In presence of the target RNA, these primers create hybrids of DIG-biotin double-labeled H1–H2, and the final product is added to LFA. Nanoparticles (NPs) of polyethylene (PE) are conjugated by streptavidin (STV) as a fluorophore. Mouse antidigoxin/DIG monoclonal Abs (mAbs) act as CPs at the TL, and biotin is immobilized at the CL. Samples from 15 Covid patients and 15 healthy individuals were gathered and tested. Using LFA, the detection time decreased from 6–8 h to 90 min. The results showed a limit of detection (LOD) around 2000 copies/mL, and a sensitivity and specificity comparable with RT-PCR and could be an acceptable replacement [77].

In another investigation, Grant and coworkers used commercially available Sars-CoV-2 Abs developed a half-strip LFA (an LFA that doesn't contain sample or conjugate pads and sample and conjugates are premixed in another container) and used an optical reader to measure the LOD. In this design, conjugates of Rockland 200–401–A50 polyclonal antibodies (pAbs) and carboxylic red latex beads (400 nm) were formed to prepare reporter probe. Polystreptavidin was used at CP at the TL and COP was goat anti-chicken (GAC) IgY. Nucleocapsid proteins of SARS-CoV-2 were tested by Genemedi and Genscript half-strips for calculating LOD. The LOD of Geneemedi and Genscript was estimated to be 0.65 and 3.03 ng/mL, respectively [78]. Furthermore, Baker and coworkers designed a LFA detection device using multivalent gold nanoparticles (Au NPs) stabilized in polymer and containing the derivatives of sialic acid with binding ability to SARS-COV-2's spike proteins. In this study, Au NPs were identified as the best target

ligand and reporting label. 2,3′-sialyl lactose-bovine serum albumin (BSA) was used as the negative control, for blocking NPs binding (Fig. 1B). The positive control was the immobilized spike protein of recombinant S1 subunit (SARS-COV-2, S1). The device was evaluated and showed that it could detect a concentration of 5 μg of virus-like particles in less than 30 min [45]. Huang and coworkers developed an IgM Ab-based LFA for detecting Sars-Cov-2 onsite. They manufactured a colloidal Au NP strip by coating the SARS-Cov-2 nucleoprotein on the membrane to form the sample capture and conjugated anti-human IgM with Au NPs to produce the detecting reporter (Fig. 1C). The detection device was evaluated and had a sensitivity of 100% and a specificity of 93.3% [46].

In an investigation, for amplifying RNA in the specimens infected by virus, Zhang and coworkers integrated direct RT-LAMP, with LFA to interpret the amplification process by optical means to detect Sars-Cov-2 in less than 40 min. In this study, the anti-fluorescein isothiocyanate (*anti*-FITC) Ab was used at the TL to act as a CP, and immobilized biotin was used at the CL. STV-coated particles were used as the labeling reporters. LAMP primers were designed to bind and amplify open reading frame (ORF1ab) and nucleoprotein genes. The evaluations demonstrated that this method has no cross-reaction with other viruses and has an accuracy of 100% in detecting the SARS-Cov-2 virus [47]. Additionally, Chen and coworkers developed a LFA *anti*-SARS-CoV-2 IgG detection device utilizing lanthanide-doped polystyrene NPs (LDP NPs). In this study, at the TL, the CP was recombinant nucleocapsid phosphoprotein of SARS-CoV-2 and at the CL, goat anti-rabbit (GAR) IgG was used (Fig. 1D). The used detector probe was prepared by functionalizing LDP NPs using rabbit IgG and mouse anti-human (MAH) IgG Ab and the whole detection process completed only within 10 min. The device's performance was compared with RT-PCR and 7 positive cases were consistent with LFA's results. One of the samples considered negative with RT-PCR but tested positive with LFA was determined to be SARS-CoV-2 IgG positive, suggesting LFA's higher sensitivity compared with RT-PCR [79].

In a research conducted by Daoud and coworkers, they used two commercially available LFA kits, including the Raybiotech and the Healgen Scientific for SARS-CoV-2 IgM/IgG, and comparatively evaluated the combination of kits and each of them alone. It was reported that the sensitivity of IgM varied between 58.9 and 66.2% and 87.7% for each kit and the combination form of kits, respectively. Still, the IgG sensitivity didn't change while using the combination strategy. Both kits showed high specificity (99.2 and 100%) [80]. Moreover, in another investigation, Azmi and coworkers developed a CRISPR-Cas13a based SARS-CoV-2 RNA detection device coupled with LFA readout integrated with a smartphone application for user-friendliness. The target RNA was amplified using CRISPR-Cas13a and RT-RPA and the final products were added to LFA for visualization. The intensity of the TL was interpreted using Fiji image J software to quantitatively detect amplified RNAs. The device showed a 98% agreement with RT-qPCR results [81].

An LFIA diagnostic test named Chinese academy of science test (CAST) was also introduced by Villarreal and coworkers for detecting IgG and IgM Abs against Sars-CoV-2 and it was applied among the Panamanian blood donors such as healthy volunteers and health care workers. They used samples from symptomatic patients and positive RT-PCR confirmed samples and then tested 19 patients with Covid-19, suspected healthy donors and health workers. The test showed a positive percent of agreement around 97.2% for the detection of both IgG and IgM. Also, this study showed that test sensitivity was associated with the number of days after the onset of symptoms, with a maximum sensitivity detected ≥15 days after the onset of post-symptoms (OPS) [82]. Additionally, Higgins and coworkers examined *anti-Sars-CoV*-2 Ab levels at various times of OPS via Easy Check Covid-19 IgM/IgG™ LFA. In this research work, 181 patients were confirmed with PCR and also collected samples from 21 donors. The obtained results demonstrated that this test's positivity is at its highest at 31–60 days of OPS. And the percentage results of IgM-/IgG^+ is somewhat equal to the outcomes of IgM^+/IgG^+ at 61–90 days of OPS, resulting from immune response shift from IgM to IgG. This study also evaluated the LFA-based method branded Easy Check Covid-19 IgM/IgG™, demonstrating their high sensitivity at 1–4 days of OPS with a sensitivity of 86% at 5–7 days of OPS [83].

In Broughton and coworkers study, the authors tried to develop *SARS-CoV-2* DETECTR, which is a CRISPR-Cas12 based on LFA to detect SARS-CoV-2 RNA extracts. The RNAs were amplified using primers that for target genes corresponding to the envelope and nucleoprotein of SARS-CoV-2 in the RT-LAMP and the final products were added to LFA for visualization. This detection device was evaluated and compared with the real-time PCR assay for SARS-CoV-2 of the U.S. CDC in analyzing samples of patients from infected by virus. Similar results with real-time RT-PCR were obtained for SARS-CoV-2 DETECTR while being rapid (~30 min), low-cost, and highly specific [84].

In another study, Peng and coworkers used a photon-counting approach integrated with LFA to develop a quantitative and sensitive test. This test utilizes the light scattered by the NC, unconjugated Au NPs, and Au NPs conjugated with SARS-CoV-2 IgG to determine Ab concentration (Fig. 1E) to develop a highly specific, low-cost, rapid, simple, and quantitative test detecting Sars-Cov-2 in clinical samples [85]. Furthermore, Van Elslande and coworkers evaluated 7 LFA-based tests of IgG/IgM Ab against Sars-Cov-2 including Clungene, OrientGene, VivaDiag, StrongStep, Dynamiker, Multi-G and Prima. The authors then compared the results with Euroimmun IgA/IgG ELISA tests in 270 samples. All seven LFA tests had a specificity of over 90% for IgM and over 91% for IgG compared to specificity of 96.1% for ELISA IgG. LFA tests had sensitivities between 92.1% and 100% for IgG at 14–25 OPS compared with 89.5% for ELISA IgG, and IgM results varied between LFA tests [86]. And also, Xiong and coworkers developed a dual-gene Sars-Cov-2 diagnostic device integrating CRISPR/Cas9 with LFA. This test simultaneously discovered the genes associated with the ORF1ab and envelope in Sars-CoV-2 RNAs, and Au NP-DNA probes were used as reporting probes. Evaluations demonstrated that this test reached 97.14% and 100% as positive predictive agreement and negative predictive agreement, respectively when the whole detection process takes under 1 h to complete. Multiple factors can cause single-gene detection methods to have false negatives; therefore, employing dual-gene detection methods can increase test reliability and versatility [87].

In conclusion, these studies demonstrated LFAs' satisfactory performance in detecting SARS-Cov-2 and can be employed for clinical applications. Different biomarkers were used for detecting SARS-CoV-2 in these researches. Multiple approaches, like integrating with other detection methods, using multiplex diagnostic LFA to improve certainty, and using other reading devices, were used to enhance LFAs further.

4.2. Influenza

Influenza viruses belong to the *Orthomyxoviridae* and cause respiratory infections. They can be transmitted by aerosol droplets from person to person or be zoonotic. Influenza causes multiple symptoms like high-grade fever, myalgia, headache, and malaise [89]. Influenza can be diagnosed via serology, Ag detection, nucleic-acid-based detection, and viral culture, RT-PCR being considered the gold standard [90,91]. In the following, various LFA-based sensing platforms regarding Influenza determination are discussed. Kawasaki and coworkers integrated a desktop

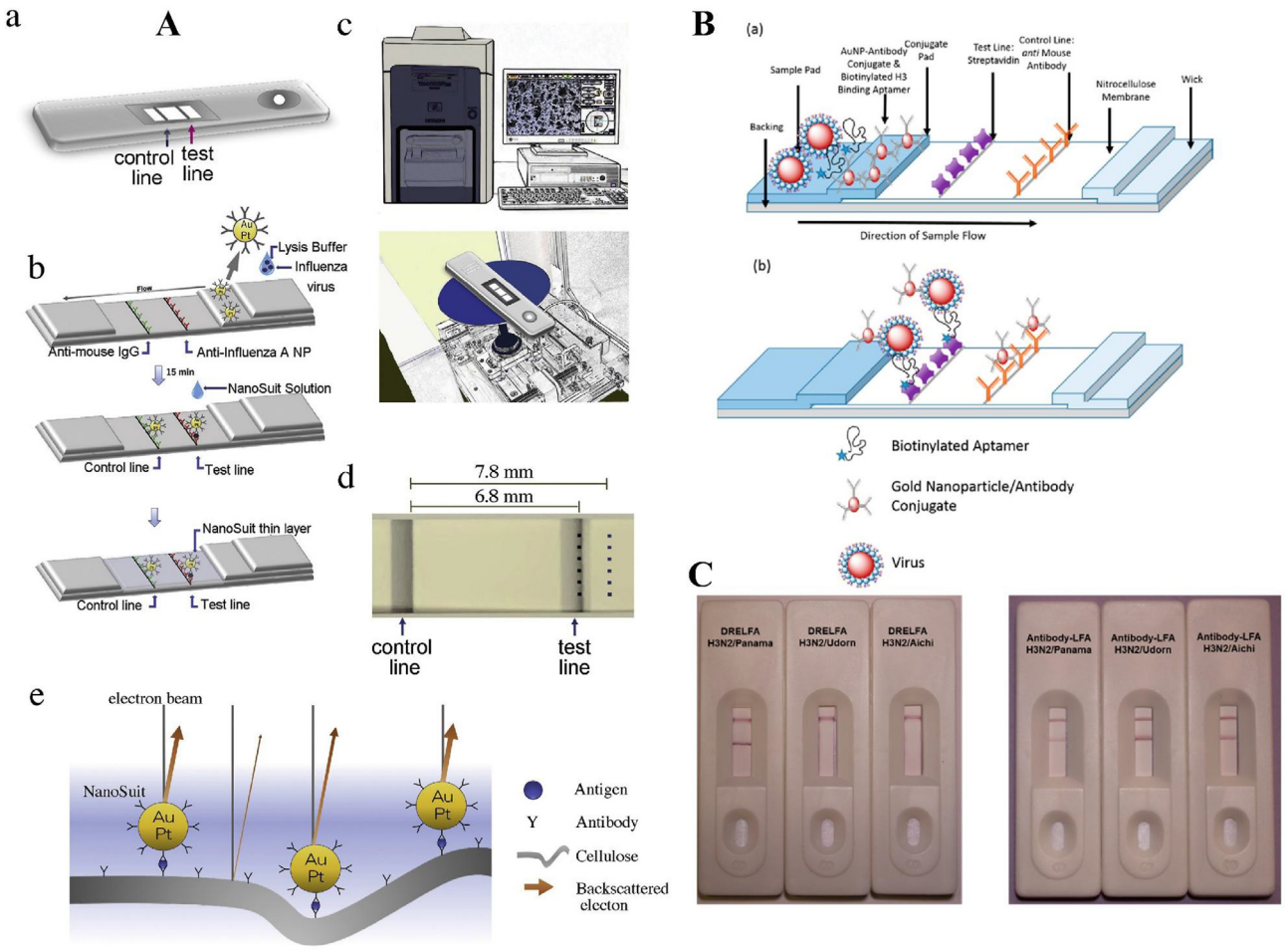

Fig. 2. (A) The TL and CL are shown on the diagnostic kit (a). The Au/Pt-Ab conjugate-linked fast LFA kit is depicted schematically (b). At the TL, the immune complex responds against nucleoprotein of influenza A, whereas at the CL, it reacts with anti-mouse IgG. (top, middle). Formation of a NanoSuit layer after NanoSuit treatment (bottom). SEM (miniscope TM4000plus; top) on a desktop (c). The placement of the kit in the SEM chamber (shown in the bottom). Establishing stations for observing in the TL and also the background (BG) regions, six fields were chosen (d). Au/Pt NPs and cellulose electron backscattering covered by a NanoSuit layer shown in the schematic picture (e). Adapted by permission from Ref. [53]. DREFLA schematic design for Influenza detection. The virus is captured by STV and is visualized by Au NPs (B). Specificity of LFA's DRELFA (C). Adapted by permission from Ref. [92].

scanning electron microscope (SEM), NanoSuit approach, and LFA to develop the LFA-NanoSuit method (LNSM) to detect the Influenza virus. In this study, gold-platinum (Au/Pt) NPs were conjugated with anti-Influenza A NP to form the reporter probe. Anti-Influenza A Ab immobilized at the TL operated as a CP, and anti-mouse IgG immobilized at the CL captured remaining NPs (Fig. 2A). The LFA results can be obtained in various ways, one of which uses the SEM to acquire high-resolution images of Au/Pt NPs to avoid limitations like cellulose swelling. This device was evaluated and reached a sensitivity of 94% and specificity of 100% [53].

In another study, Kim and coworkers developed a near-infrared (NIR) to NIR upconversion NPs (UC NPs)-based LFA detecting Avian Influenza virus (AIV) during 20 min. In this study, Ca-coated UC NPs were conjugated with *anti*-AIV NP Abs to form the reporter probe. Anti-AIV NP Ab was also immobilized at the TL to act as a CP, and the immobilized goat anti-mouse (GAM) IgG at the CL was used a control probe. The excited UC NP emitted signals that can be read and interpreted by NIR-to-NIR UC NP-based LFA for detecting HPAI H5N6 and LPAI H5N2 viruses. Evaluating this device revealed LOD of 102 and 103.5 EID50/mL for LPAI H5N2 and HPAI H5N6, respectively. This LFA's sensitivity is 10-fold higher than commercial LFAs and unlike them, the detection process isn't disrupted by the opacity and brown color of stool samples [54]. For detecting the H5N2 Influenza virus, Kim and coworkers employed a pair of aptamers to develop an LFA. In this study, systematic evolution of ligand by exponential enrichment (SELEX) and graphene-oxide (GO) were used and an assay based on the fluorescence resonance energy transfer (FRET) mechanism and GO (GO-FRET) were utilized to manufacture virus particle-specific aptamers with capability to bind different parts of virus particles at the same time. Au NPs were used as signaling labels and were conjugated to JH4APT. J3APT was used at the TL as a CP, and poly-A sequence was immobilized at the CL as the COPs. Upon interpreting by ImageJ program and after evaluating the results, the LOD being 1.27×10^5 and 2.09×10^5 EID50/mL was determined for buffer and the duck's feces, respectively [93].

Another LFA approach utilizing surface-enhanced Raman scattering (SERS) phenomenon for simultaneously detecting human adenovirus (HADV) and IAV H1N1 has been designed by Wang and coworkers. Fe_3O_4@Ag magnetic NPs (MNPs) in this study were coated by Ag, and dye molecules of DTNBs were attached to it. Then HADV Ab or H1N1 Ab was conjugated with it to form the reporter probes. LFA contained two TLs; one used HADV Ab and the other H1N1 Ab, and a CL sprayed with polyclonal goat anti-mouse (pGAM) IgG. The SERS signals emitted from test and CLs then can

be read to detect Ags quantitatively. This device was evaluated and showed a LOD of 50 pfu/mL for H1N1 and ten pfu/mL for HADV [94].

In another investigation, a dual recognition element LFA (DRELFA) to highly sensitive detection multiple strains of Influenza have been developed by Le and coworkers. In this study, specific Abs were manufactured and screened using SELEX and were conjugated with biotin. Au NPs were also conjugated with Influenza mAbs, and in the presence of Ags, both the aptamer and the Au NPs would detect the virus and form an immune-complex (Fig. 2B and C). The STV immobilized at the TL would capture the immune-complex, and the Au NPs of the immune-complex would act as reporters. The rest of the Au NPs were captured at the CL by anti-mouse Abs. This device was evaluated and demonstrated a LOD of 2×106 virus particles, and the quantitative results, using the TL color intensity, showed a significant correlation with RT-PCR results [92].

Furthermore, Park and coworkers developed a CRISPR-Cas12a-based LFA to detect IAV and Influenza B (IVB). In this research, RNAs were amplified using DNA endonuclease-targeted CRISPR trans reporter (DETECTR) combined with recombinase polymerase amplification (RPA) and LAMP. Primers were manufactured to detect IAV matrix and IBV hemagglutinin genes for amplification. The amplified RNAs were added to an LFA, and the visualization process took about 2 min, with a LOD of 1×100 PFUs per reaction [95]. Also, Ma and coworkers integrated RPA and lateral flow dipstick (LFD) to detect IAV H7N9. RPA was used to amplify RNA employing primers that were manufactured to detect H7 and N9 genes. The final amplified RNAs were then added to two LFDs (one for H7 and the other for N9 detection) to be visualized and detected by the naked eye. Both LFDs demonstrated 100% sensitivity and showed no cross-reaction with different Influenza subtypes [96].

Bai and coworkers developed magnetic quantum dot nanobeads (MQDNBs) based LFA for detecting IAV in clinical samples. Magnetic $MnFe_2O_4$ nanobeads were conjugated with quantum dots (QDs) and *anti*-IAV Abs to form the reporter probes. The Abs of IAV sprayed on the TL acted as CPs, and pGAM IgG immobilized at the CL captured remaining MQDNBs. In the presence of Ag, the MQDNB-Ag complexes were arrested at the TL. After excitation, emitted fluorescence signals were collected by the smartphone CMOS image sensor and were analyzed to detect IAV quantitatively. This LFA's LOD was measured to be 22 pfu/mL, and the whole detection process took up about 35 min [97].

An oseltamivir hexylthiol (OHT)-based LFA to detect Tamiflu-resistant Influenza virus was developed by Hwang and coworkers. OHT has a higher affinity to Tamiflu-resistant Influenza viruses compared with Tamiflu-susceptible ones. In this study, Au NPs were conjugated with OHT and were used as reporter probes. In the TL, *anti*-AIV nucleoprotein was used as a CP, and Tamiflu resistant NPs at the CL captured OHT-conjugated Au NPs. With the accumulation of Tamiflu-resistant on the TL, a purple line becomes visible to the naked eye (Fig. 3A). This LFA was evaluated and demonstrated a LOD of 5×10^2 pfu, which is suitable for clinical application [55].

Sun and coworkers developed an RPA-LFA to detect IAV and IBV simultaneously. In this investigation, primers that detected IAV were conjugated with fluorescein isothiocyanate (FITC) and biotin, and IBV-detecting primers were labeled with DIG and biotin. The Influenza virus TNA was amplified using RPA, and these primers and the final products were added to LFA. Au NPs were conjugated with STV to form the detecting probes. LFA strips consisted of two TLs sprayed with *anti*-FITC and anti-DIG, and BSA immobilized captured remaining Au NPs at the CL. The formed lines were visible by the naked eye, and a smartphone can capture and interpret the images of the lines to quantitatively detect Influenza RNAs. This device reached sensitivities of 78.57% and 87.50% for Influenza A and B, respectively. The LODs were also determined to be 500 and 50 copies per reaction for Influenza A and B, respectively [99]. In another study, a SERS-based LFA has been developed by Zhang and coworkers to simultaneously detect 11 respiratory tract infection pathogens, including IAV and IBV. In this study, Au NPs were conjugated with Raman Dyes of either Nile blue A (NBA) or methylene blue (MB), and of the 11 pathogens capture nucleic acids (Fig. 3B). The LFA contained 6 T dots, 5 of which included two sets of capture nucleic acids, and one of them had one, and control nucleic acids were immobilized at the CL to capture the remaining Au NPs. A SERS reader can quantitatively detect emitted signals and distinguish between the Raman dyes; therefore, the captured Ags can be distinguished by their specific Raman dyes and their T dot position on the LFA. This LFA was evaluated and showed excellent specificity, and IAV and IBV LOD were determined to be 0.031 pM and 0.035 pM [100].

The design of a carbon nanotag-based LFA for detecting IAV was reported by Wiriyachaiporn and coworkers. In this study, carbon nanostrings were conjugated with BSA and mouse mAb anti-Influenza A nucleoprotein forming detector probe. Mouse anti-Influenza A mAb nucleoprotein was also used at the TL as a CP, and the immobilized GAM IgG captured remaining carbon NPs. The intensity value of the TL was interpreted by Image J program to detect IAV quantitatively. The whole detection process took about 15 min, and LOD was calculated to be 350 TCID50/mL [101]. Also, a horseradish peroxidase (HRP)-based LFA has been introduced by Zhang and coworkers to detect IAV and IBV simultaneously. HRP was conjugated with 11F12 and 10B6 *anti*-IAV and IBV mAbs to form the detector probes. The LFA strip contained two TLs, with capture Abs immobilized on them, and a CL that used GAM IgG. This device was evaluated and showed sensitivities of 77.5% and 71.2% and specificities of 99.8% and 99.8% for IAV and IBV [100].

These studies showed that LFAs have a satisfactory performance in detecting the influenza virus. Using multiplex LFAs is a great way to diagnose common subtypes of influenza and avoid under treatment. Different approaches like integrating with other detection devices and alternate reading devices were used to improve LFAs performance.

4.3. Human immunodeficiency virus (HIV)

HIV is a member of the *Retroviridae* family that attacks the host's CD_4^+ lymphocytes, leading to acquired immunodeficiency syndrome (AIDS) [102]. A third of the people infected with HIV never notice it until it has advanced to a more advanced state and remain an asymptomatic career, which increases the burden of the disease [103]. Therefore, HIV infection should be diagnosed early and needs rapid and cheap POCT for diagnosis and surveillance [104]. Accordingly, Tang and coworkers employed a dialysis method to increase sample concentration in LFA testing to improve its sensitivity. The dialysis device is comprised of a semi-permeable membrane and a glass membrane containing PEG. The LFA device was designed to detect HIV nucleic acid in 15 min, using Au NPs as reporter labels, and conjugated with an HIV detection probe. Evaluations demonstrated that using this method the LFA sensitivity increases 10-fold compared to conventional LFA with a LOD of 0.1 nM [105].

On the other hand, a multiplex LFA to detect HIV, Hepatitis C virus (HCV), and Hepatitis A virus (HAV) simultaneously has been introduced by Lee and coworkers. In this study, diagnostic probes were constructed by conjugating viral Abs to manufacture proteinticles that can attach to multiple Abs to increase sensitivity. Au NPs were conjugated with protein A and BSA to form labeling probes. There were three TLs and one CL on LFA strips; the HIV TL consisted of proteinticles conjugated with the TL of PHIV-1 and

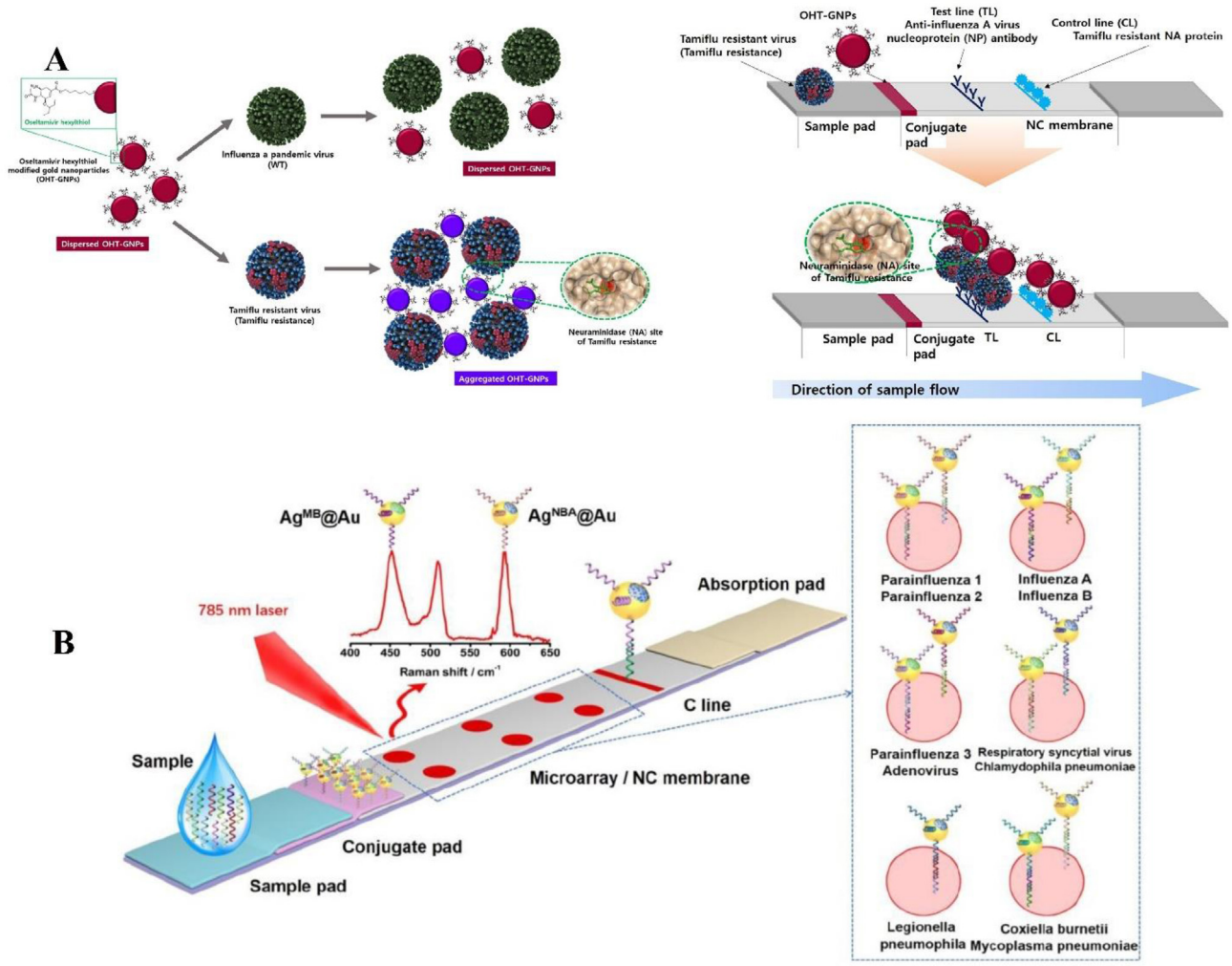

Fig. 3. Schematic diagram of the colorimetric method for detecting Tamiflu resistance by OHT-modified Au NPs (OHT-Au NPs). Due to the OHT and anti-trimeric fluorescent virus combination, the color of OHT-Au NPs changed from dark red to purple (A). Adapted by permission from Ref. [55]. Schematic diagram of SERS LFM for nucleic acid detection of 11 RTI pathogens with RD-encoded SERS core-shell nano-tags (B). Adapted by permission from Ref. [98].

PHIV-2, HAV consisted of proteinticles conjugated with PHAV-1, PHAV-2, and PHAV-3, and HCV TL consisted of proteinticles conjugated with PHCV-1 and PHCV-2. The immobilized GAH Abs at the CL captured remaining Au NPs. The formed TLs can be detected by the naked eye or be processed by line analyzer and image analysis software to detect quantitatively. This device was evaluated and reached a sensitivity and specificity of 100% [57]. On the other hand, Calvaria and coworkers worked on a multimodal LFA to detect HIV1 and HIV2 Abs, consisting of only one TL. In this study, AuNPs emitted red lights and were conjugated with gp36 antigen that detects HIV-1 Ab, and gold nano stars emitted blue lights and were conjugated with gp41 antigen that detects HIV-2 Ab. Immobilized gp36 and gp41 were also used at the TL, and avidin was used at the control line. The color of the test line determined the detected HIV subtype, and the evaluations showed that this approach improved LFA's performance [106].

Moreover, Fu and coworkers developed a SERS-based LFA to detect HIV-1 DNA quantitatively. Raman reporter Malachite green isothiocyanate (MGITC) and oligonucleotides as detection DNA to form reporter probes were used. Immobilization of captured DNAs and Control DNAs were performed at the TL and CL of the LFA strip. A Raman instrument reader can be used to interpret Raman intensity to detect HIV-1 DNA quantitatively. This device was evaluated and showed LOD around 0.24 pg/mL, which was 1000-fold greater than the typical LFAs [58]. In another study using QDs, Deng and coworkers developed fluorescence LFA to recognize HIV DNA quantitatively. In this study, QDs conjugated with hairpin DNA were used as reporter probes, t-DNA and C-DNA were immobilized at the TL and CL. Evaluations demonstrated that the LOD was 0.76 pM and was more than satisfactory for clinical application [59].

A magnetic immunochromatography-based LFA to detect HIV-1 and HIV-2 using multibranched peptides (MBPs) has been investigated by Granade and coworkers. Magnetic beads conjugated with protein A were used as reporter probes. Immobilized HIV-1 and HIV-2 MBP Abs were used at the TL, and protein A acted as the COP in the CL. A magnetic reader was used to measure TL activities on minutes 20 and 40 to detect HIV-1 and HIV-2 quantitatively. This LFA's results were concordant with the WB reference test and showed an acceptable reproducibility [107]. In another research, Granade and coworkers developed an LFA to detect recent or long-term HIV-1 simultaneously. In this study, Au NPs conjugated with protein A were used as reporter probes, and protein A was also used as a COP at the CL. Multisubtype gp41 recombinant protein (rIDR-M) was immobilized at two TLs with two different concentrations to differentiate between recent and long-term

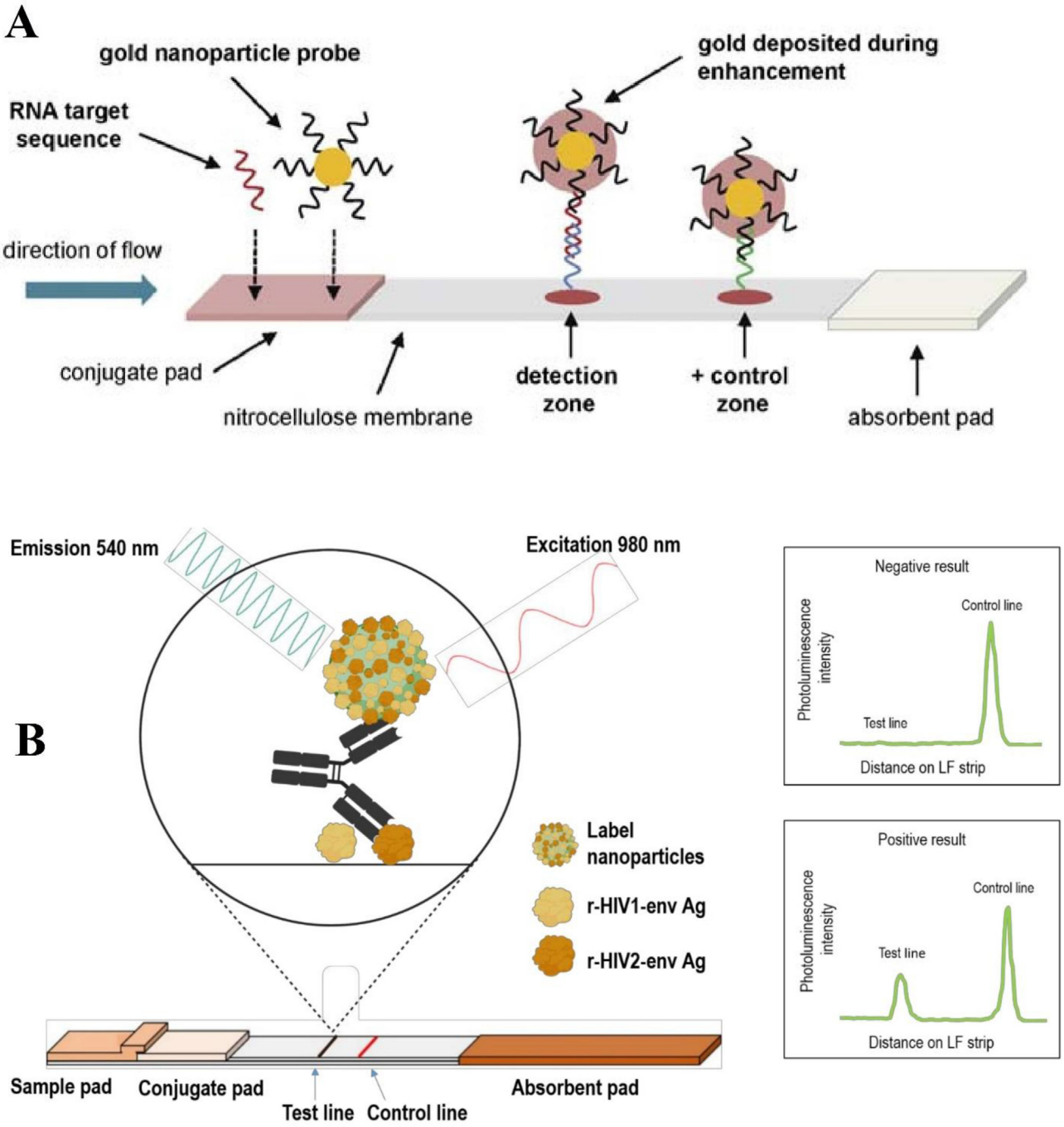

Fig. 4. Schematic diagram of quantitative LFA for HIV-1 RNA detection (A). Adapted by permission from Ref. [109]. UC NP antiHIV1/2 strip design and dual Ag bridging test principle. Recombinant HIV1 and HIV2 envelope Ags are used as captures on the TL and coupled to the surface of UC NP to detect *anti*-HIV Abs. UC NP is excited at 980 nm, and the emission peak intensity at 540 nm indicated a negative or positive reading (B). Adapted by permission from Ref. [110].

infections. In recent infection, only the TL with the higher rIDR-M concentration will detect HIV, while both TLs will visualize long-term disease. This device was evaluated and showed 95% agreement with the fabricated assay [108].

An integrated nucleic acid sequence-based amplification (NASBA) with LFA for quantitatively detecting HIV-1 RNA was designed by Rohrman and coworkers. Au NPs conjugated with detector nucleic acids that bind to the HIV gag gene and form reporter probes (Fig. 4A). Capture sequences immobilized at the TL act as CPs, and control sequences at the CL bind with the remaining Au NPs. To increase sensitivity, HIV RNAs are amplified by NASBA and are added to LFA. Image analysis was used to interpret TL intensity to detect amplified RNA quantitatively. This sensing assay was evaluated and demonstrated a LOD of 9.5 log10 RNA copies, which is suitable for clinical application [109].

Bristow and coworkers evaluated the commercial SD BIOLINE HIV/Syphilis Duo test enjoying LFA method for detecting HIV and Syphilis. The participants consisted of 298 GHESKIO clinic attendees who were 18 years or older, and 237 of them were female, of which 49 were pregnant. This LFA reached a sensitivity of 99.2%

and a specificity of 97.0%. In pregnant women, the sensitivity was 93.3%, and specificity was 94.1% [111].

Also, thermal contrast amplification (TCA) approach was reported to be useful in improving the LFA detection sensitivity against HIV p24 protein. In this research, Au NPs were conjugated with *anti*-p24 mAb to form reporter probes. Anti-p24 pAbs were CPs at the TL, and p24 recombinant protein was immobilized at the CL to capture the remaining Au NPs. An infrared (IR) camera was used to measure temperature changes in Au NPs resulted from laser irritations. The IR signals at the CL represented the specific binding (SB), and the emitted IR signals from the TL demonstrated the non-specific binding (NSB). Binding ratio (BR) can be obtained by the BR= SB/NSB, and is an indicator of Au NPs distribution and can be used to detect p24 quantitatively. Evaluations showed this LFA's LOD is 8 pg/mL, which is lower than conventional LFAs and is close to ELISA LOD [112].

UC NP reporters to develop a double Ag LFA introduced by Martinskainen and coworkers luminescent were also used for the detection of Abs against HIV-1 and HIV-2 in 30 min (Fig. 4B). In this study, UC NPs were conjugated with recombinant envelope glycoproteins of HIV-1 (r-HIV-1env) and −2 (r-HIV-2env) to form reporter probes. The Ags corresponding to r-HIV-1env and r-HIV-2env were also immobilized at the TL to act as CPs, and HIV-1 gp41 rabbit pAb was used at the CL to capture the remaining UC NPs. The captured UC NPs at the TL and CL were excited with an IR laser. An Upcon reader device detected the emitted signals from excited UC NPs, and SAS JMP Pro 14 statistics software interpreted the signals to detect Abs of HIV-1 and HIV-2 quantitatively (Fig. 4B). This device was evaluated and reached a sensitivity of 96.6% and a specificity of 98.7% [110].

Another investigation performed by Zhang and coworkers, demonstrated an LFA by fixing cotton threads onto a membrane to detect HIV. In this study, the hydrophilicity of cotton threads was decreased using Hexadecyltrimethoxysilane (HDTMS) and was fixed paper to form the LFA's membrane. Cotton-embedded papers delay fluid flow and therefore increase the reagents' interaction and, in turn, the LFA's sensitivity. Au NPs were conjugated with detector probes, and test probes and COPs were immobilized at the TL and CL. Employing cotton threads increases LFA's sensitivity four-fold compared with conventional LFAs [98].

To detect HIV p24 Ag, A NIR fluorescent microsphere immunochromatography-based LFA was developed by Qi and coworkers. Fluorescent microspheres were conjugated with Dylight 800 and label Abs to form reporter probes. GAM IgG Ab was immobilized at the CL to capture the remaining NPs and HIV-1 P24 Ab was immobilized at the TL to act as a CP. Portable fluorescent scanner KY-100 then detected and interpreted emitted signals from TL and CL to detect HIV p24 Abs quantitatively. This device was evaluated and demonstrated a LOD of 3.4 pg/mL, which is suitable for clinical application [113].

Using multiplex LFAs helps physicians to simultaneously detect various types of HIV and other sexually transmitted viruses. Different methods like using alternate readers and reporter probes and adjusting membranes were used to improve LFAs quality. The alternate readers also helped to detect HIV biomarkers quantitatively.

4.4. Hepatitis B virus (HBV)

HBV as a leading causes of liver disease in the family *Hepadnaviridae* can be transmitted sexually or via blood transfer. HBV can cause both acute and chronic hepatitis and can lead to liver cancer [114,115]. There are several serum markers for HBV, including HB Abs, HB Ags, HBc Ab, and HBe, each of which is found during different phases of HBV replication [116]. HBV infection can be asymptomatic but can still be transferred, and early diagnosis should be applied using POCTs [117]. By using QDs nanobeads, a multiplex integrated fluorescent LFA to detect infectious pathogens including HIV, HCV, HBV, and Treponema pallidum (TP) was developed Rong and coworkers. In this study, multicolor QDs nanobeads were used as labeling reporters to increase sensitivity and specificity. Different Ags for HIV-1/2, HCV, HBV, and HBs were used both as capture and detection probes. The GAM IgG Ab was used at the CL to detect and capture remaining QD nanobeads. An optical receiver and an Android app were used to receive and interpret fluorescent signals emitted from QD nanobeads at the TL to diagnose and quantify the captured pathogen. This LFA was evaluated, and pathogen detection sensitivities varied between 88.33 and 93.42%, and specificities were between 87.67 and 93.15% [60].

In another investigation, Srsomwat developed an automated electrochemical LFA (eLFA) to detect HBV quantitatively. In this study, pyrrolidinyl peptide nucleic acid (acpcPNA) as an immobilized detector was used at the TL. The used membrane in this device had a unique design, allowing the tested serum to go through a non-delayed channel and the HBV DNA present in the serum to rapidly bind and hybridize with acpcPNA. Then a fluid containing Au^{3+} (which is used for the metallization process) would go through a delayed channel and reach the TL after the DNA hybridization has occurred. Au^{3+} binds to hybridized DNA, and after the electrodeposition process, an electrical signal demonstrates HBV DNA detection (Fig. 5A). The whole detection process takes 7 min and has a LOD of 7.23 pM [118].

Choi and coworkers integrated polydimethylsiloxane (PDMS) barrier and a paper-based shunt with an HBV-detecting LFA device to enhance the assay's sensitivity. Au NPs were used as reporter labels, and STV was used at the TL. Employing a PDMS barrier and a paper-based shunt delays fluid flow, thus, enhances the reagents' interaction chance and eventually leads to signal enhancement. The evaluations demonstrated that this method allows sensitive detection of HBV (~10^2 IU/mL) while also retaining the rapidity of LFA tests [119]. Moreover, an LFA to detect HBV DNA was developed by Gao and coworkers. In this study, Au NPs coated with STV and Au NPs labeled probes so-called detProbe-Au-single stranded DNA (ssDNA) were used as amplifying reporters. The synthesized T-DNA and C-DNA at the TL and CL were immobilized (Fig. 5B). Evaluation of such device presented a LOD of 0.01 pM [61]. Another study demonstrated a LFA sensing assay using Au NPs by Kim and coworkers to detect HBsAg. Different sizes of Au NPs and their efficiency in various pH value and Ab concentration was investigated. UV−vis spectroscopy was used to analyze and optimize Au NPs conjugation, and it was established 42.7 nm. Au NPs performed best under multiple circumstances in detecting HBsAg [121].

In addition, Gong and coworkers developed an up-conversion NP-based LFA (UC NP-LFA) procedure for the detection of multiple targets, including HBV. Four different probes including UC NPs and UC NPs-DNA and UC NP-Ab were used in this study. A UC NP-LFA reader calculating the optical density of the TL and background was used to obtain analyte concentration. This LFA was evaluated by testing multiple analytes and correlated with the gold-standard method in testing HBV DNA [122]. Li and coworkers also developed an up-converting phosphor technology-based LFA (UPT-LFA) to rapidly and quantitatively detect HB surface antibody (HBsAb). In this study, up-converting phosphor particles conjugated with HBsAg were used as reporter probes, and HBsAg was immobilized at the TL, and goat *anti*-HBsAg Ab was loaded at the CL. The UPT-LFA was evaluated and compared with Abbott Axsym AUSAB and conventional ELISA, reaching a good correlation with ELISA, making it a sensitive and rapid quantitative test for detecting HBV [123]. Furthermore, another UC NP-LFA to detect HBsAg has been

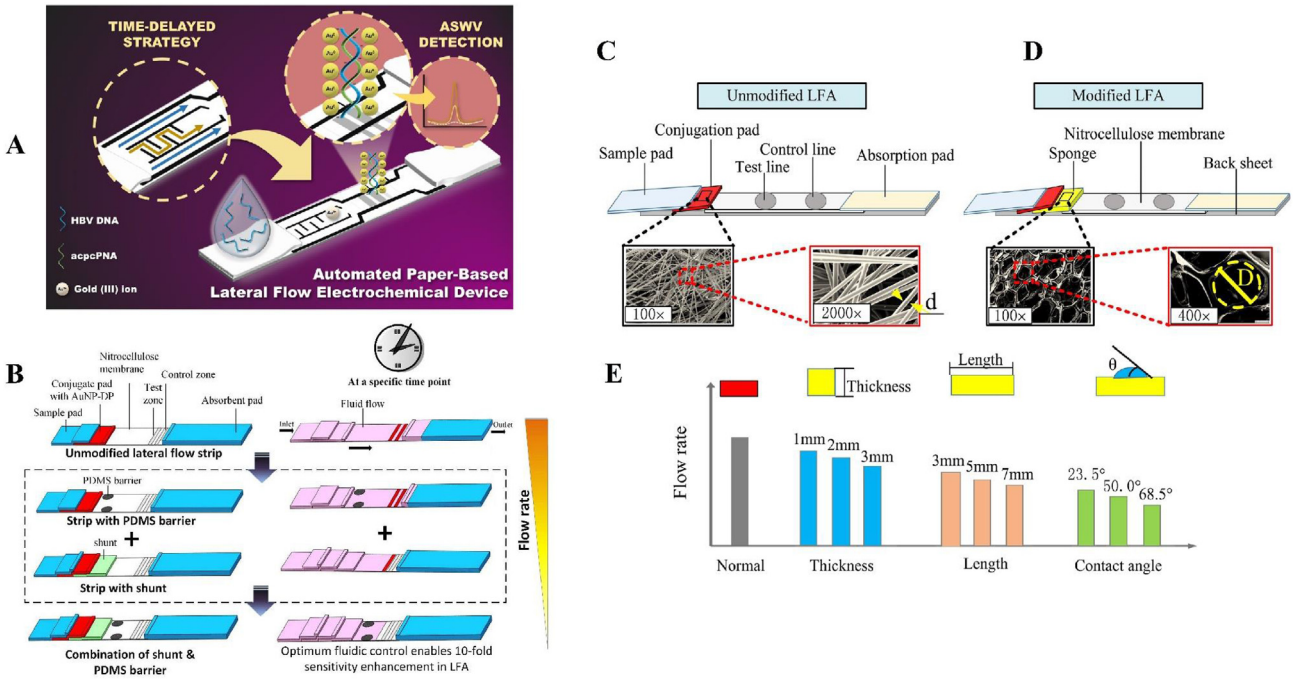

Fig. 5. The unique design of eLFA membrane for delaying the fluid flow (A). Adapted by permission from Ref. [118]. The effect of adding PDMS and shunt to LFA alone and simultaneously on fluid flow rate and sensitivity (B). Adapted by permission from Ref. [119]. The demonstration of unmodified LDA (C) modified LFA (D) and the sponge shunt optimization parameters (E). Adapted by permission from Ref. [120].

developed by Martiskainen and coworkers. In this investigation, UCNPs conjugated with *anti*-HBsAg mAb were considered as reporter probes. Immobilized *anti*-HBsAg mAb at the TL was used and the CL consisted of rabbit anti-mouse IgG. This UC NP-LFA was evaluated and compared with a conventional LFA. UC NP-LFA reached a sensitivity of 95.4%, while the conventional LFA's sensitivity was 87.7% [62].

An automated fluorescent LFA to detect HBsAg and HCV Ab was also reported by Ryu and coworkers. In this research, fluorescent europium chelates [Eu (III)] conjugated with HBsAg or HCVAg or mouse *anti*-HBsAg mAbs were used as reporter probes. A fluorescence scanner was used to detect and quantify captured analytes at the TL. The device was evaluated and reached sensitivities of 99.8%, 100%, and 99.8% for HBsAg, *anti*-HBs, and *anti*-HCV and specificities of 99.3%, 100%, and 99% for HBsAg, *anti*-HBs, and *anti*-HCV tests [55]. On the other hand, Si and coworkers employed a signal amplification system (SAS) to develop an LFA to detect HB e-antigen (HBeAg). In this study, Au NPs conjugated with Ab used as reporter probes and capture Abs labeled with biotin were used as CP. In the presence of HBeAg, the reporter probe and CP would form a dendritic complex. Dendritic complexes would be captured at the TL. The SAS-LFA was evaluated and demonstrated LOD of 9 ng/mL and displayed a complete match with ELISA [124].

Furthermore, Tang et al. demonstrated a hybridization-based LFA by combining a sponge shunt with conventional LFA to detect HBV nucleic acid. Au NPs were used as labeling reporters. A sponge was integrated with an LFA strip to delay fluid flow, increase the interaction time and test sensitivity (Fig. 5C, D and E). The modified LFA was evaluated and reached a LOD of 10^3 copies/mL, in contrast with conventional LFA, which had 10^4 copies/mL [120].

An integrated betaine-assisted recombinase polymerase assay (BRPA) with an LFA to detect HBV DNA was introduced by Yi et al. This study showed that in recombinase polymerase assay, nonspecific amplification occurs and the addition of betaine suppresses unwanted nonspecific amplifications. This test was combined with LFA to allow naked-eye detection. The evaluations revealed that 1000 copies of HBV DNA could be detected in a 50 μL mixture using this device with a sensitivity and specificity of 90% and 100%, respectively [125].

An integrated polymerase spiral reaction (PSR) with LFA for sensitive and rapid determination of HBV DNA was developed by Lin and coworkers research group. In this investigation, final products of PSR were labeled with FITC probe and biotin taken to LFA for visualization. STV conjugated Au NPs were used as LFA labeling NPs. Anti-FITC mAb as a capture probe was used at the TL, and biotin-BSA was immobilized at the capture line, and the LFA visualization process takes up to 5 min. This sensing platform was examined and showed a specificity of 100%, which is higher than the qPCR and LAMP assay. LOD was measured 5.4 copies/mL that is ten times more sensitive compared with qPCR and LAMP [126].

LFAs can be more than helpful in developing countries with endemic areas for HBV and lack the expensive conventional devices for HBV detection. Multiplex LFAs are effective in hepatitis viral detection because they also detect various types of hepatitis virus and other sexually transmitted viruses. Integrating LFA with other detection methods, and adjusting the membrane improved sensitivity. These studies also examined alternate readers and reporter probes in quantifying and enhancing detection.

4.5. Dengue virus (DENV)

DENV belonging to the family *Flaviviridae* is transmitted via *Aedes* mosquitoes. Dengue disease symptoms vary from asymptomatic fever to more severe symptoms like hemorrhagic fever and shock [127,128]. Dengue fever is prevalent in 112 countries, around half of the world population is considered at risk and is estimated to have 390 million incidence cases per year [129,130]. Dengue fever occurs in underdeveloped urban areas and needs a cheap and portable POCT for diagnosis [130]. In the following, some recent advances in LFA sensing platforms are comprehensively explained

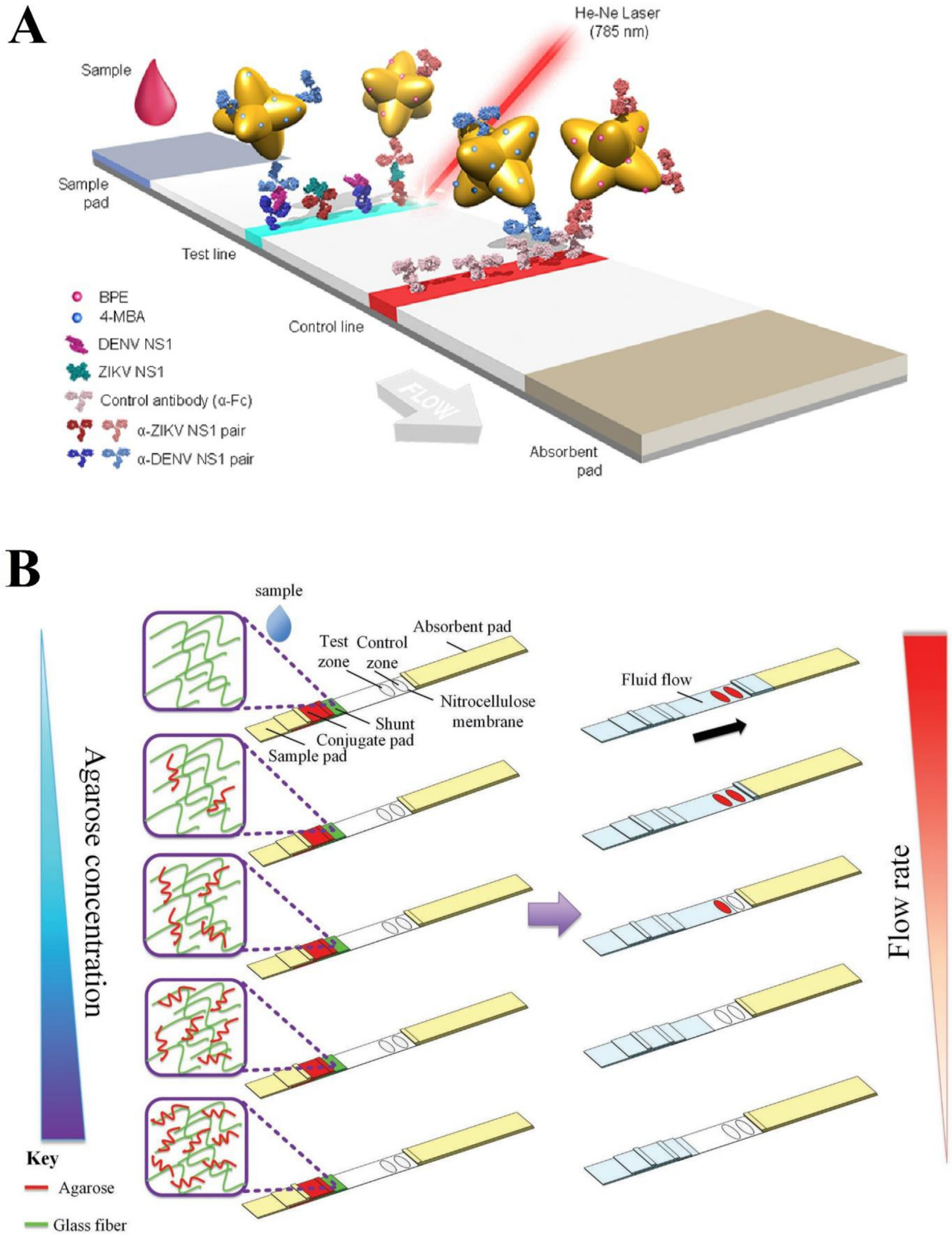

Fig. 6. Schematic diagram of DENV and ZV multiplexed detection using SERS-based LFA (A). Adapted by permission from Ref. [132]. Schematic design of agarose based-LFA to delay fluid flow for DENV detection (B). Adapted by permission from Ref. [133].

regarding the DENV. For example, Jeon et al. compared SERS-based LFA strips with conventional LFA to detect mosquito-borne viruses like ZKV and DENV. LFA strips that can resist at high temperatures are needed for these endemic viruses detection in tropical regions. Unlike conventional LFA strips that employ Au NPs, the SERS-based LFA strips in this study used Au NPs conjugated with silica shells as reporter labels to withstand sensitivity loss in higher temperatures. Evaluations demonstrated that conventional LFA strips LOD significantly increase with an increase in temperature, but SERS-based LFA strips LOD value remains the same [131]. Additionally, another SERS-based LFA for detecting and distinguishing the NSP1 of ZKV and DENV was established by Purra et al. In this research, different nanotags were used to differentiate between two viruses. In SERS-encoded gold nanostars, (GNSs), 1,2-bis(4-pyridyl)ethylene (BPE) was conjugated to an *anti*-ZKV Ab to form GNS-BPE-*anti*-ZKV, so-called "Z-nanotag", and 4-mercaptobenzoic acid (MBA) was conjugated to GNSs and *anti*-DENV Ab to form GNS-MBA-*anti*-DENV or called "D-nanotag". The calculated surface enhancement factors could differentiate between nanotags (Fig. 6A), increasing the specificity of the test while also decreasing the LOD 15- and 7-fold for ZV-NSP1 and DENV-NSP1, respectively [132].

Additionally, Axelrod et al. employed a new approach in LFA to detect and quantify DENV NSP1. Target-specific Ab-conjugated to HRP are present in the conjugation area, and NSP1 molecules are immobilized in the capture area. In negative serums that don't contain NSP1, *anti*-NSP1-HRPs will be captured by immobilized NSP1 in the capture area, and don't reach the last layer, thus do not generate any signals. However, NSP1 Ags present in positive serums will bind to *anti*-NSP1-HRPs to form NSP1-specific Ab-HRP immune complex, thus preventing them from being captured by immobilized NSP1s at the capture area, allowing the immune complex to reach the last layer to bind with HRP substrate, generating the signal. This novel LFIA was evaluated and was determined to be five-fold more sensitive than a gold standard ELISA test [134]. Also, Kumar et al. developed an LFA diagnostic test for rapid detection (10 min) of DENV NSP1. In this investigation, Au NPs conjugated with reduced GO (Au NPs-rGO) were used as reporter labels and were conjugated with FITC-NSP1 Abs, increasing the sensitivity and achieving a LOD of 4.9 ng/mL [135]. Tran and coworkers developed a magneto-enzyme LFIA to detect DENV NSP1. In this research, GAM Ab at the CL was used, and at the TL, capture Ab was used. In addition, biotinylated, mAb-conjugated magnetic beads were used as reporter probes. This assay was evaluated and reached a sensitivity and specificity of 100%, demonstrating no cross-reaction with other viruses and LOD between 0.1 and 1 ng/mL applicable for different DENV serotypes [64]. Additionally, a screen-printed gold electrode (SPGE) combination with an LFA strip in the development of an electrochemical lateral flow immunosensor (ELFI), a diagnostic device for DENV was introduced by Sinawang and coworkers. In this device, NSP1 Ab conjugated with immunonanobeads would bind to sample DENV NSP1. The formed immunocomplex would attach with immuno-conjugated electrodes, emitting an electrical signal, allowing a quantitative detection with LOD of 0.5 ng/mL [136].

In another investigation, a NIR fluorescent dye-based LFA detecting *anti*-DENV1 IgG Abs by Purra et al. was fabricated. This investigation demonstrates that DYlight-800 coated GAH IgG Abs were used as sample capture, and recombinant protein from envelope of DENV1 at the TL was used. This device was evaluated and compared with DENV Duo IgG/IgM cassette and Panbio DENV IgG ELISA. NIR-LFA reached a sensitivity of 95%, which was the same with compared tests. However, testing diluted serum samples, NIR-LFA had a lower LOD compared with these tests [63].

Choi et al. combined agarose hydrogel into the glass fiber shunt to develop a paper-hydrogel hybrid LFA test for detecting the DENV. Using this hybrid allows fluid control while maintaining paper-based advantages, increasing Au NP-detector probes (Au NP-DPs) and DENV RNA interaction (Fig. 6B), thus improving device sensitivity [133].

An LFA for simultaneous detection of DENV and Chikungunya virus (CHIKV) IgM/IgG using two-color detection labels was established by Lee and coworkers. This study used blue labels for *anti*-IgG and red labels for *anti*-IgM for both CHIKV and DENV. Two TLs, one consisting of CHIKV Ag and one consisting of DENV Ag, captured virus-specific Abs, while anti-goat IgG was used to capture all Abs in the CL. Captured Abs in the TLs emitted blue or red lights, resulting in a mixture of blue and red light emitted from the TL of virus, captured and calculated to determine IgG and IgM concentration [137].

Yrad and coworkers developed an Au NP-based LFA to detect DENV-1 RNA in less than 20 min. In this investigation, a capture probe specific to DENV-1 (dDNA) was immobilized at the TL to detect DENV-1 RNA and generates a red light, and a control probe (cDNA) was immobilized at the CL to detect dextrin-capped Au NP. The whole detection process took about 20 min, and LOD was calculated to be 1.2×10^4 pfu/mL [65]. Xiong and coworkers integrated reverse transcription recombinase-aided amplification with LFA to detect DENV RNA in blood samples. In this study, primers were designed to detect and amplify DENV RNA and were labeled with FAM and biotin. The amplified nucleic acids were then added to commercially available lateral-flow dipsticks (USTAR, Hangzhou, China) to determine the results. This approach was evaluated and showed a LOD of 10 copies/μL, with no cross-reactivity with other pathogens [138].

LFAs can also help detect DENV in underdeveloped areas. These areas also may have high temperatures that need devices that can withstand environmental inconveniences. These studies examined the impact of using alternate readers and reporter probes in quantifying and improving viral detection. Adjusting the membrane also increased the LFA's sensitivity.

4.6. Human papillomavirus (HPV)

HPV belongs to the *Papovaviridae* family and is a sexually transmitted virus that causes genital warts [139]. Some subtypes such as 16 and 18 showed high risk and may lead to malignancies [140]. Cervical cancer caused by HPV has a 500,000 incidence rate annually and 250,000 mortalities [141]. Different strategies and approaches are proposed and applied for HPV screening [140]. LFA can be an excellent tool for rapid diagnosis and can be used for screening purposes. For instance, Grant et al. developed a LFA monitoring method to assess HPV vaccination status by detecting *anti*-HPV16 Abs. In this study, Au NPs conjugated with GAH IgG were used as reporter probes. The LFA had three TLs, spotted with different dilutions of HPV16 L1 VLPs in PBS to discriminate between different serum Ab concentrations, and human IgG was immobilized at the CL. This method was evaluated by testing 28 serum samples, 15 of which received two or more vaccine doses, and 13 received one amount or none. The test could discriminate between these two groups well and could be used two assess patients' vaccination status [142].

In another investigation, a combination of a miniaturized PCR with LFA to detect HPV DNA has been demonstrated by Liu et al. HPV-nucleic acids were amplified by the IR-COCONUT PCR platform, and the final products were added to LFA for visualization. In this LFA, anti-DIG alkaline phosphatase (AP) conjugate was used as a detector probe, STV was immobilized at the TL and GAR IgG was used at the CL. The LFA visualization process takes up to 25 min, and digital images taken from the TL could be interpreted by computer software to detect PCR products quantitatively [120].

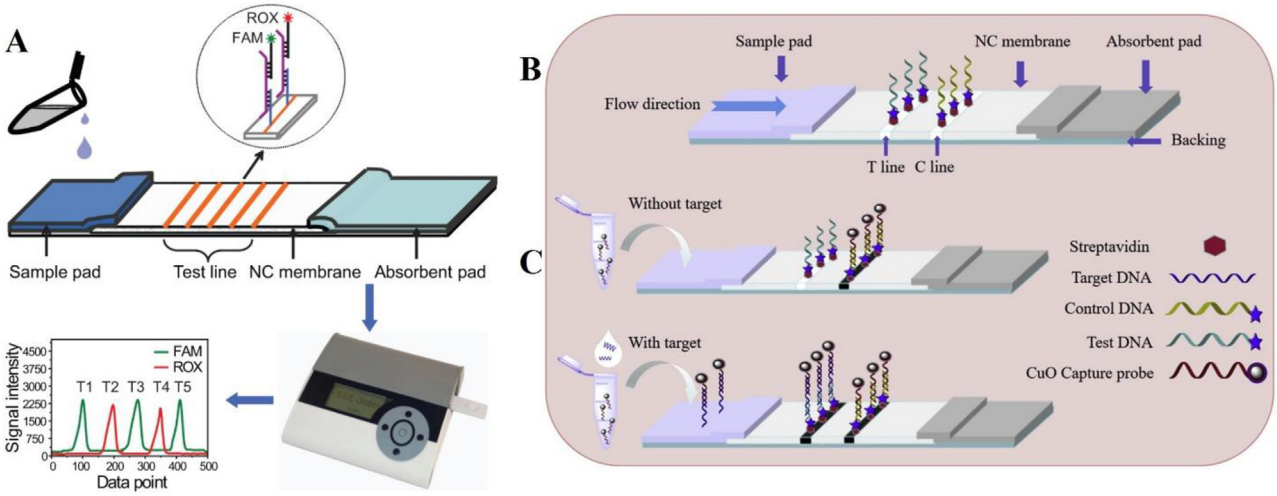

Fig. 7. Schematic design of fluorescence LFA for multiplex HPV detection (A). Adapted by permission from Ref. [143]. Schematic illustration of the CuO-based LFSB for HPV detection showing flow direction (B) and the detection procedure (C). Adapted by permission from Ref. [144].

Also, Wang et al. integrated Antarctic thermolabile uracil-DNA-glycosylase (AUDG) with nucleic acid amplification (NAA) techniques and LFA to simultaneously detect HPV16 and HPV18. This research work demonstrates that AUDG-NAAs were used to amplify nucleic acids (HPV16 E7 and HPV18 L1) and labeled with FITC/biotin or DIG/biotin, and the final products were added to LFA for visualization. In this LFA, STV-coated dyed polymer NPs (SA-DNPs) as labeling particles were used and at the first TL, *anti*-FITC was immobilized. At the second TL, anti-DIG was immobilized. Biotin-BSA forms a firm binding with STV and therefore was used as chromatography control. The final device was evaluated and demonstrated a LOD of 5×10^5 copies and showed no cross-reactivity between HPV16 and HPV18 [66].

Fluorescent probe-based LFA to detect 13 types of HPV simultaneously have been effectively fabricated by Xu and coworkers. This study explains that primers conjugated with fluorophore were used to amplify HPV nucleic acids, and the final products were added to LFA for detection and discrimination. The LFA reader had two-channel color detection; therefore, each line could be conjugated with two different coloring labels, and the probes on the seven lines each were conjugated with FAM or ROX. The probes were designed to detect 13 types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and capture probe including human β-globin (HBB) was used as a control probe (Fig. 7A). The LFA visualization process takes up to 30 min. This device was evaluated, and the results showed LODs of 102 copies/μL for HPV type 18 and 10 copies/μL for HPV types 6, 11 and 16. No cross-reactivity was reported, and there was an agreement of 98% with the reverse dot blotting kit (GenoArray) [143].

Finally, Yang et al. developed copper oxide nanoparticles (CuO NPs)-based LFSB to detect HPV 16. In this study, CuO NPs conjugated with STV and capture DNA were used as CPs. At the TL and CL, test and control DNAs were immobilized and at the TL or CL, the captured CuO NP-SA-DNAs would form a brown stripe that can be detected qualitatively by the naked eye (Fig. 7B and C), or quantitative detection can be performed by adding washing buffer and using KinBio strip reader software to interpret line color intensity [144].

LFAs are rapid devices that can be great for HPV screening. Using multiplex LFA devices is necessary for screening multiple HPV subtypes. Also, using different readers help to quantify the detection.

4.7. Zika virus (ZKV)

As a member of the *Flaviviridae* family, ZKV like DENV is commonly transmitted by mosquitos [145]. ZKV infection causes signs and symptoms like fever, arthralgia and myalgia, conjunctivitis, headache, and rash and may cause Guillain-Barré syndrome [146]. Laboratory tests like serological assays, molecular assays, virus isolation, and IHC can be used to detect ZKV [147]. An integrated RT-LAMP with LFA to develop a device for ZKV detection was reported by Lee et al. ZKV RNAs in this research were amplified with RT-LAMP using primers conjugated with DIG, and the final products were added to LFA for visualization. Au NPs conjugated with STV and anti-DIG were used as reporter probes. Amplified ZKV RNAs contained DIG-conjugated primers and were detected by detector probes and were also captured by immobilized anti-DIGs at the TL. Biotin was also affixed in the CL to bind with STV at the Au NPs [148].

In another study, Rong et al. established a smartphone-based LFIA to detect NSP1 in ZKV. In this study, QD microspheres were conjugated with ZKV NSP1Ab and BSA to act as reporter probes. ZKV NSP1 Ab was also immobilized at the TL to capture detected antigen complex, and GAM IgG pAbs at CL was also used to capture QD microspheres. The captured Ag complex at the TL emitted fluorescent can be detected and interpreted by smartphone and its camera, and the whole detection process takes about 20 min. This device was evaluated and showed limited cross-reactivity with similarly structured DENV, and LOD in serum was calculated to be 0.15 ng/mL [149].

Xu et al. developed fluorescent carbon dots (CDs)-based LFA to detect the ZKV. In this investigation, CDs-assembled were loaded into dendritic SiO_2 to form fluorescent CD-based silica (FCS) spheres (Fig. 8A and B). The FCS spheres were conjugated with ZKV-01 Ab and BSA to act as reporter probes. In the presence of ZKV, FCS spheres form a complex, which is captured at the TL by another *anti*-ZKV Ab, which emits blue light under UV lamp. The remaining FCS spheres migrate to the CL and capture with GAM IgG. This device was evaluated and compared with a conventional Au NP-based LFA. The results showed that this device had a LOD of 10 pg/mL, and had its sensitivity was 100-fold higher than conventional LFA [150].

In another study, polycaprolactone (PCL) electron-coating NC membrane to develop a nucleic acid-based LFA for detecting ZKV

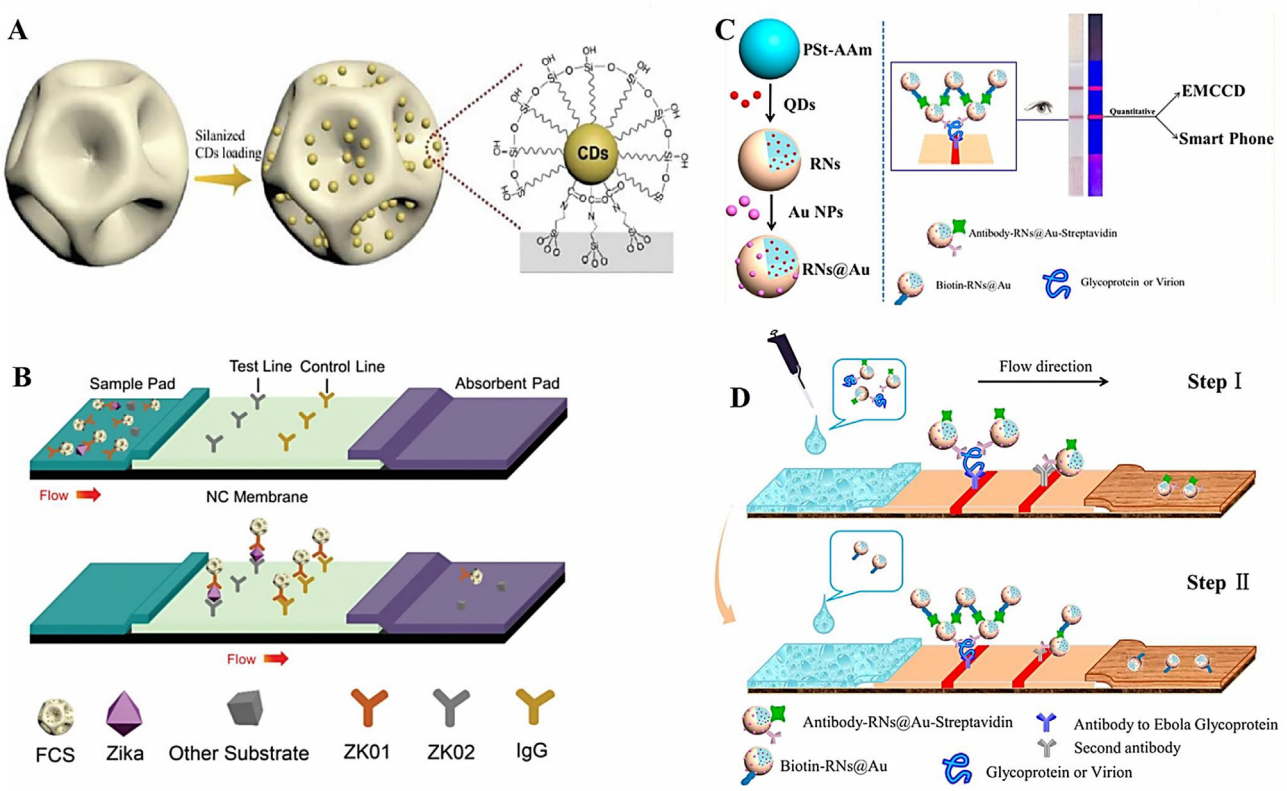

Fig. 8. The synthetic processes for CD-embedded SiO_2 spheres are depicted in a schematic diagram (A). Schematic illustration for detection of Zika NS1 protein using the iFCS-based LFA (B). Adapted by permission from Ref. [150]. Schematic design of dual-signal readout LFA for EV detection (C). Two-Step Detection of EBV with RNs@Aubased LFA (D). Adapted by permission from Ref. [68].

was introduced by Yew and coworkers. In this study, Au NPs conjugated with detector probes acted as labeled detectors. Electrospun-coated membrane delays the fluid flow and increases interaction between analyte and reagents. Evaluations demonstrated that this device could detect ZKV cDNA with a LOD of 0.5 nM, and its sensitivity is 10-fold higher than unmodified NC membrane-based LFA [151,152].

4.8. Ebola virus (EV)

EV belongs to the *Filoviridae* family and causes a hemorrhagic illness called EV disease transmitted via blood and other body fluids [153]. Its signs and symptoms include fever, chills, malaise, anorexia, severe headache, and myalgia [154]. Many laboratory tests are available for EV detection, but due to its infection nature, POCTs are beneficial for early detection and prevention. In this way, Hu et al. developed a rapid, sensitive, and quantitative LFA to detect the EV glycoproteins (GPs). In this study, multifunctional nanosphere (RNs@Au) was used as sample capture. Signal enhancers consisted of RNs@Au with Ab and STV-conjugated on it. These reporters binding with EV glycoprotein were captured by *anti*-EV glycoprotein at the TL. They emitted two signals, fluorescence signal for quantitative detection interpreted by smartphone and colorimetric signal detectable by the naked eye (Fig. 8C and D). Another set of RNs@Au was conjugated with biotin and developed to bind with STV to amplify signals. This assay allowed the detection of 2 ng/mL glycoprotein by the naked eye in less than 20 min [68]. Additionally, an LFA employing a smartphone reader to detect *anti*-EV IgG semi-quantitatively was developed by Brangel et al. In this research, anti-human IgG conjugated with Au NPs were used as reporting probes, and recombinant EV proteins were used at the TL. In addition, a smartphone app was developed to acquire semi-quantitative results from the detected IgG at the TL. The device was evaluated and reached a sensitivity of 100% and specificity of 98%, making it a decent test for clinical application [155].

On the other hand, Feuerstein and coworkers developed a NIR light-emitting fluorescent-based LFA for detecting the (GPs of EV. Fluorescent nano-diamond particles (FNDPs) conjugated with *anti*-EV GPs were used as labeling reporters. In addition, another set of *anti*-EV recombinant GPs at the TL were immobilized. And, GAH IgG at the CL was immobilized to capture the remaining FNDPs. The captured FNDPs emitted NIR light that could be interpreted by an optoelectronic device [156]. Phan et al. evaluated an LFA for EV detection that developed by the Naval medical research center. Au NPs were used as labeling reporters, and *anti*-EV Ags were conjugated to them. Polyclonal *anti*-EV Ag was used at the TL as capture probes, and anti-goat IgG was used to capture remaining reporters at the CL. The LFA was tested and compared with RT-PCR using 290 plasma samples and 237 oral samples. The LFA reached a sensitivity of 87.9% and specificity of 96.8%. EZ1 real-time PCR achieved a sensitivity of 96.7% and specificity of 92.6% [157].

4.9. BK virus (BKV)

BKV infects humans in early childhood and remains there as an asymptomatic infection, and in appropriate circumstances, like immunosuppression, BKV reactivates and begins its pathogenicity process [158]. BKV infection leads to nephropathy; therefore, it is more common in kidney transplant receivers

immunocompromised to avoid graft rejection, so BKV detection is crucial in these patients [159,160]. To detect BKV and cytomegalovirus (CMV) in post-transplantation for early infection detection Kaminski et al. developed CRISPR-Cas13-based assay integrated with LFA readout. In this test, BKV DNA and CMV DNA are isolated from urine and blood samples. In addition to this, CXCL9 messenger RNA, a graft rejection marker, is detected in urine samples. This device was integrated with a commercially available LFA for detection optimization, and the final products were added to it. The integrated device was evaluated and showed high sensitivity and specificity for BKV, CMV, and CXCL9 messenger RNA detection [161].

In another study, nucleic acid-based LFA has been established by Huang et al. to detect human polyomavirus BKV in 45 min. In this study, oligonucleotide probes were manufactured and were conjugated to Au NPs to form DPs and CPs that detected different parts of DNA. In the presence of the Ag, the target DNA is captured by both CP and DP. Rabbit polyclonal *anti*-STV Ab was used as TL and captured the Au NP-DP-target-Au NP-CP immune-complex and formed a red line. Rabbit polyclonal *anti*-BSA Ab was immobilized at the CL, capturing the remaining Au NPs. The line intensity was interpreted by image software to calculate the quantitative results. The assay LOD was evaluated to be 10^7 copies/mL, and this test presented no cross-reactivity with other polyomaviruses [162].

4.10. Norovirus

Norovirus belongs to the *Caliciviridae* family and causes winter vomiting disease [163]. This disease can be asymptomatic or be presented with fever, vomiting, and diarrhea and can be transmitted person-to-person or via food infections [164]. To develop an LFA to detect norovirus, Hagström and coworkers used phage NPs. In this study, Norwalk (norovirus GI.1) virus-like particles (VLPs) were synthetically manufactured and purified. SAM-AviTag M13 phage was cultured and conjugated with BSA and *anti*-norovirus mAb to be used as reporter probes. Anti-norovirus Abs at the TL were immobilized to detect noroviruses, and *anti*-M13 Abs were used at the CL to capture remaining M13 phage. This device was evaluated and compared with Au NP-based LFA. The results demonstrated that M13 phage-based LOD is 107 for VLPs per mL, 100-fold lower than Au NP-based LFA [165]. Moreover, Doerflinger and coworkers developed a nanobody-based LFA to detect norovirus. The Nanobody used in this study bound to a region on norovirus capsids and was conjugated with biotin and Au NP. After the addition of norovirus particles, STV on the TL capture immune complexes resulting from nanobodies. This device was evaluated and reached a sensitivity of 80% and specificity of 86% [166].

4.11. Severe fever and thrombocytopenia syndrome virus (SFTSV)

A phlebovirus is known to be the cause of SFTS showing symptoms of gastrointestinal disorders, leukocytopenia, and fever [167]. SFTSV belongs to Bunyaviridae family and can be transmitted via ticks [168]. SFTSV can be diagnosed using real-time PCR, ELISA, and virus isolation, and POCT like LFA can be employed for early detection [169]. In Xu et al. investigation, they developed a fluorescence-based LFA to detect SFTSV. In this study, carbon dots/SiO_2 nanospheres (CDSNs) were conjugated with *anti*-SFTSV mAbs to act as reporter probes. Anti-SFTSV Abs were also immobilized at the TL to capture detected Ags, and GAM IgG Abs at the CL were used to capture remaining CDSNs. This device was evaluated, and the SFTSV detection results were consistent with PCR showing no cross-reactivity with HCG, AFP, CEA, CA125 [170]. In another research, Zuo and coworkers developed an LFA using Au NPs conjugated with *anti*-SFTSV mAbs as reporter probes. These mAbs at the TL were immobilized to CPs, and GAM IgG at the CL was used for capturing Au NPs. This device was evaluated with a LOD of 1 ng/mL, and the whole detection process took up to 10 min [71].

4.12. LFA sensing assays for determination of other types of viruses

LFA-based devices have also been effectively used in the determination of other kinds of viruses. In an investigation, Li and coworkers constructed an Au NP-based LFA using IgM Abs for detecting four TORCH pathogens including herpes simplex virus type 2 (HSV-2), CMV, rubella virus and toxoplasmosis. In this research, the μ-chain of MAH IgM mAb (anti-human IgM) was conjugated to AuMag to form the reporter probes. TORCH Ags were immobilized at the TL to act as CP, and the GAM IgG Ab at the CL was used as a CP. Serum samples of 162 infected individuals were tested by this device and 100% sensitivity, specificity with no occurrence of cross-reactivity were reported [67]. In another study, Zhu and coworkers developed a fluorescence-based LFA to detect Epstein-Barr virus (EBV) and HPV16. In this study, carboxyl-modified fluorescent microspheres were used as labeling reporters, and NC substrate strips were used as a membrane. BSA and whole human IgG Ab were used as negative and positive controls, respectively. EBV nuclear Ag-1 (EBVNA-1) was immobilized at the TL to capture EBV Ags. This device was again modified to study *anti*-HPV 16 E7 IgG detection using recombinant HPB16 E7 proteins. The results showed a 100-fold improvement compared with previous studies that used a glass substrate [171].

Additionally, HSV-2 is tried to serologically detect by the development of a LFA device in Goux and coworkers research. In this investigation, persistent luminescent NPs (nanophosphors) of strontium aluminate conjugated with GAH pAbs were used as labeling reporters. At the TL, recombinant gG2 Ag of HSV was immobilized to detect *anti*-HSV Ab, and GAH IgG capture Ab was used at CL to capture the remaining nanophosphors. Nanophosphors at the TL reacted to light and excited, emitting light that can be detected and interpreted by an iPhone app to quantitatively detect *anti*-HSV Abs captured at the TL [172].

In an investigation reported by Townsend and coworkers, the authors evaluated commercially available Tetracore Orthopox BioThreat® LFA assay for detecting Orthopoxvirus (OPVs), Vaccinia virus (VACV), and Monkeypox virus (MPXV). In this device, Au NPs conjugated with *anti*-VACV Abs were used as reporter probes. The *anti*-VACV Abs were also immobilized at the TL to capture detected OPVs. This assay reached a LOD of 107 pfu/mL and showed a false positive in one out of 11 non-OPV samples; therefore, it is applicable for screening and detecting OPVs [173]. In Rebollo and coworkers study, the authors developed an immune-chromatographic LFA to detect the West Nile virus (WNV). In this study, 1D11 (a mAb that detects WNV's envelope glycoprotein) was conjugated with red carboxyl-latex particles to act as reporter probes, and BSA was conjugated with blue latex particle to serve as a control complex. 1D11 was also immobilized at the TL to capture Ags detected by the detector probe, and the CL consisted of mAbs that captured control-complex proteins. After 10 min, in the presence of WNV, a red line is formed at the TL, informing the test positivity. This test was evaluated and successfully detected different WNV strains with LOD of 105 TCID50/mL and showed no cross-reactivity with other flaviviruses [174].

In recent years, for the effective management of viral diseases, hundreds of POCTs with high sensitivity and specificity have been developed and are commercially available now for detection of most pathogenic viruses such as SARS-Cov-2, HIV, HBV, HCV, Influenza, Ebola, Dengue, HPV, Zika, HCV and etc (Table 2).

Table 2
Some of commercially available LFA strips for fast detection of infectious viruses.

LFA device	Company name	Detected Virus	Detection time
SARS-Cov-2 Antigen Rapid Test Cassette (swab)	Spring healthcare	SARS-Cov-2	15 min
E25Bio's SARS-CoV-2 Direct Antigen Rapid Test (DART)	E25bio	SARS-Cov-2	15 min
Encode SARS-CoV-2 Antigen Rapid Test Device	Zhuhai Encode Medical Engineering Co.,Ltd	SARS-Cov-2	20 min
SureScreen COVID-19 Rapid Antigen Test Cassette	Surescrenn diagnostics	SARS-Cov-2	15 min
Statu s™ COVID-19/F lu	Lifesign	SARS-Cov-2/Influenza A and B	15 min
SARS-CoV-2 & Influenza Antigen Combo Rapid Test Kit	MedOmics	SARS-Cov-2/Influenza A and B	15–20 min
SCREEN TEST COVID-19+FLU A/B	ScreenItalia	SARS-Cov-2/Influenza A and B	15 min
SARS-CoV-2 & Influenza A/B & RSV Antigen Combo Test Kit	Shenzhen Microprofit Biotech Co., Ltd	SARS-Cov-2/Influenza A and B/RSV	15 min
QuickProfile COVID-19 ANTIGEN Test	LumiQuick Diagnostics Inc.	SARS-Cov-2	15 min
quickvue influenza a+b test	Quidell	Influenza A and B	10 min
rapidSTRIPE H1N1	Analytik Jena	Influenza A H1N1	5 min
Sofia Influenza A+B FIA	Quidell	Influenza A and B	15 min
Xpect Flu A&B Kit	REMEL INC	Influenza A and B	15 min
OSOM™ Ultra Flu A and B Rapid Test	Sekisui Diagnostics	Influenza A and B	15 min
Influenza A&B test	Nanoentek	Influenza A and B	15 min
OraQuick® In-Home HIV Test	OraSure Technologies	HIV 1 and 2	20 min
autotest VIH®	AAZ-LMB	HIV 1 and 2	15 min
BioSURE HIV Self Test	BioSURE United Kingdom Ltd	HIV 1 and 2	15 min
INSTI HIV Self Test	bioLytical Laboratories	HIV 1 and 2	5 min
Atomo HIV Self-Test	Atomo Diagnostics	HIV 1 and 2	15 min
Aware™ HIV-1/2 OMT Oral HIV Self Test	Calypte Biomedical Corporation	HIV 1 and 2	20 min
Exacto® HIV Screening Test	Biosynex Group	HIV 1 and 2	10 min
INSTI HIV-1/HIV-2 TEST	Biolytical	HIV 1 and 2	1 min
DETERMINE HBsAg 2	Alere Medical Co. Ltd	HBV	15–30 min
iCare Hepatitis B Home Test Kit	iCare	HBV	15 min
SCREEN TEST EPATITE B − HBsAg	ScreenItalia	HBV	15 min
Hepatitis B Rapid diagnostic test	Maccura Biotechnology Co., Ltd.	HBV	10 min
SD BIOLINE HCV	Abbott Laboratories	HCV	15 min
OraQuick® Ebola Rapid Antigen Test	OraSure technologies	Ebola virus	30 min
ReEBOV™ Antigen Rapid TestKit	Corgenix Medical Corporation	Ebola virus	15 min
Panbio™ Dengue Duo Cassette	Abbott Laboratories	Dengue virus	15 min
Dengucheck WB	Adiwara Worldwide	Dengue virus	15 min
Dengue Rapid Test	Maternova inc	Dengue virus	10 min
SD BIOLINE Dengue Duo (Dengue NS1 Ag + IgG/IgM)	Abbott Laboratories	Dengue virus	15 min
OncoE6™ Cervical Test	Arbor Vita Corporation	HPV 16 and 18	15 min
StrongStep® HPV 16/18 Antigen Rapid Test	Liming bio	HPV 16 and 18	15 min
AVantage HPV E6 Test	Hologic	HPV 16/18/45/33/58/52	15 min
Zika SimPlex	E25bio	Zika virus	15 min
One Step Zika IgG/IgM Antibody Test	Maternova inc	Zika virus	15 min
Biopand Zika NS1 Rapid Test	Biopanda reagents	Zika virus	15 min
DPP® Zika IgM Assay USA	ChemBio diagnostics inc.	Zika virus	15 min
Duo HSV-1/2 IgG/IgM Rapid Test CE	CTK Biotech	HSV 1 and 2	10 min
iCare Herpes-2 Home Test Kit	iCare	HSV-2	15 min
Herpes Simplex Virus 1 & 2 Blood Test 5 Test Pack	Home health UK	HSV 1 and 2	15 mimutes
AmpliVue HSV 1 + 2 Assay	Quidell	HSV 1 and 2	Not mentioned

5. Conclusions and future outlooks

This review mainly focused on investigating LFAs in virus detection. Conventional LFAs are rapid, user-friendly, and accessible but certain limitations like low sensitivity and showing the results just by an on/off format constrain them for wide application in virus testing. In addition to these, some LFAs suffer from low reproducibility. The final result and detection time depend on various factors like biomolecule affinity, sample nature, fluid viscosity, and surface tension. The analytes must be in solution form, and samples should be fluid; therefore, pretreatment is required for non-fluidic samples, which is time-consuming. Using gold, silver, and other nanoparticles may limit shelf life and increase test costs [175]. However, many LFAs investigated in this study employed novel methods and strategies to overcome these limitations, but other approaches can further improve LFA performance. Immobilizing Abs on the LFA strip can be organized by passive absorption and covalent coupling; the latter conjugates Abs to carboxyl groups on functionalized nanoparticles that improve performance. The LFA strip membrane can also be adjusted to delay fluid flow and increase reagents interactions to ensure that reagents binding is optimal, increase sensitivity and reduce detection time. Using dissolvable barriers between the conjugate pad and test line and between test line and control line has been proven effective in increasing LFA's sensitivity [176,177]. Integrating LFA with other methods can significantly increase its sensitivity. Many techniques like LAMP, PCR, CRISPR-Cas13a, RT-RPA, etc., were used to amplify virus nucleic acids and employed LFA for visualization. With the recent advancements in nanomaterials, many approaches have been suggested to improve LFAs. Two sets of GNPs with two different sizes can be used as reporter probes, as the GNPs with smaller sizes bind to the analytes, and the GNPs with bigger sizes bind to the first group of GNPs [178]. Using alternate labeling reporters, like magnetic particles, quantum dots, upconverting phosphors, europium, latex beads, enzymes, and colloidal carbons could also enhance the signal and increase LFA's sensitivity. Several undesirable interface issues can occur in developing LFAs at molecular levels like in NP-antibody conjugates, immobilized antibodies, and Antibody-biomarker interactions, and with the advancements in nanotechnology, resolving these interface issues can be the next breakthrough in LFA design [179]. Also, the employment of alternative reporters allows novel visualization methods that can be digitized and allows quantitative detection. Visual detection, luminescence detection, magnetic detection, SERS

detection, electrochemical detection was used in LFAs, each having its advantages and disadvantages. Quantitative detection can also be obtained using image analysis software that calculated the virus concentration by interpreting the line intensity at the TL. Multiplex detection was also used at these LFAs, some detecting 13 different Ags simultaneously, which can help confirm the differential diagnosis in infectious diseases with similar signs and symptoms. Subsequently, LFAs can be widely used in clinical settings and replace expensive and time-consuming methods with these moderations. Finally, this review finds that LFAs as one of the effective alternative methods in virus detection, mainly due to their rapidity and portability. By further improvements, they can be commonly used in clinical diagnosis.

Declaration of competing interest

The authors have no conflict of interest to declare.

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