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Review

LFA: The Mysterious Paper-Based Biosensor: A Futuristic Overview

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Abstract: Lateral flow assay (LFA) is emerging as one of the most popular paper-based biosensors in the field of the diagnostic industry. LFA fills all the gaps between diagnosis and treatment as it provides beneficial qualities to users such as quick response, Point-of-care appeal, early detection, low cost, and effective and sensitive detections of various infectious diseases. These benefits increase LFA's dependability for disease management because rapid and accurate disease diagnosis is a prerequisite for effective medication. Only 2% of overall healthcare expenditures, according to Roche Molecular Diagnostics, are spent on in vitro diagnostics, even though 60% of treatment choices are based on this data. To make LFA more innovative, futuristic plans have been outlined in many reports. Thus, this review reports on very knowledgeable literature discussing LFA and its development along with recent futuristic plans for LFA-based biosensors that cover all the novel features of the improvement of LFA. LFA might therefore pose a very significant economic success and have a significant influence on medical diagnosis.

Keywords: LFA; history; parts; types; construction; futuristic applications

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1. Introduction

Point-of-care (POC) biosensors are widely used for medical diagnosis and the detection of food toxicity and chemical contamination. Biochemical markers can be quickly detected with requested sensitivity and selectivity. Recently, the ASSURED criteria for POC devices and their functional requirements were outlined by the World Health Organization (WHO), including that they are equipment-free, affordable, sensitive, specific, user-friendly, rapid, and robust [1]. There are various biosensors developed based on POC criteria such as wearable, portable, and implantable biosensors [2–4]. Among these, LFA is considered the easiest to operate without any specialization required in the context of POC devices. Due to its low cost, abundance, biodegradability, and disposability compared to plastic and metallic components, paper has become a particularly popular component for POC devices. Additionally, due to its distinctive porous structure, which can assist fluid migration without the need for an external power source, paper itself possesses special separation and "pump" properties. Paper can be designed to perform a variety of tasks, including wettability, permeability, and reactivity, making it a useful "smart" material for biosensor construction [5]. Paper-based biosensors have shown to be transportable and do not require any other reagents or equipment, nor do they require any further energy source. Paper substrates are widely used in the diagnostic field for the detection of diseases; for example, Brazaca et al. [6] used a paper substrate for the detection of Alzheimer's. Recently, Hasan et al. [7] constructed different patterns of paper electrodes to detect the dengue virus with the help of an aptasensor. Lateral flow assay is among the best illustrations of portable paper-based technology. A specific kind of biosensor called a lateral flow device (LFD) fabricates the recognition layer onto the surface of a porous membrane. It is performed over a strip with several components integrated on a plastic substrate. Paper-based lateral

flow assay is one POC technology that satisfies the aforementioned requirements and has experienced rapid technological development for numerous applications [8–10] mainly for environmental and medical purposes, especially in low-resource areas [11]. Over 2 billion LFAs are produced annually, with over 400 million produced specifically for HIV and malaria tests [12]. A new study demonstrated that LFA-based biosensors can yield a much better LOD and can be used as systems for multiplexing diagnosis [13]. Furthermore, in recent years, research has shown that such paper-based diagnostic power can be amplified with the support of smartphone methods, and their availability is increasing in the evolving world. As paper-based methods continue to develop, our cooperative determinations should report the key continuing trials of commercialization and translation [14]. There are various scientific literatures based on LFA that have been reported on by prominent researchers, such as Parolo et al. [15], who summarized very impactful tutorial-based articles that include all aspects of LFA construction. In another review, Mak et al. [16] very systematically presented overall general information associated with LFA. Other than this, the history of LFA was also evaluated by Miocevic et al. [17] and O'Farrell B [18], while the different applications based on LFA were beautifully illustrated by Di Nardo et al. [19]. The present article describes advanced and innovative LFA-based applications such as cotton-thread-based LFA, LFA-On-Drone (L-O-D), Electronic-LFA (e-LFA), and paper-based LFA, encompassing all elements of LFA supplemented with recent references. It also exploits the general report on lateral flow assay as a unique and reliable paper-based biosensor along with a brief history and description of components, design, and principles, which are discussed first. Following this, the architecting of LFA and the advantages and disadvantages of LFA are presented. This is followed by different types of LFA, which were also classified. Finally, the paper concludes with a discussion of applications in this field along with some suggestions for future research possibilities.

1.1. History of Lateral Flow Assay

Lateral flow assay is considered one of the most modern and trending techniques, but this is not completely accurate as it is an ancient method of diagnosis that has been used since the 1940s. Firstly, LFA is derived from paper chromatography, which was invented by Martin and Synge in 1943 and refined by Consden, Gordon, and Martin in 1944. After 1945, there was a boom of work in this field [20,21]. In different countries, researchers used this method to develop diagnostic kits using different samples. As one example, the ancient Chinese were among the very first people to use saliva-based diagnostics. Saliva was frequently used as rapid evidence of guilt in customary practices. One of the first written accounts of a pregnancy diagnostic test based on urine can be found in ancient Egyptian literature. The latex agglutination test, the first of which was invented in 1956 by Plotz and Singer, in which they produced a paper device to detect glucose (semi-quantitatively) in urine for the first time, served as the technological foundation for lateral flow immunoassay. Different LFA-based immunoassays were also developed in the same year, including Plate-based immunoassays. Berson and Yalow developed the first radio-immunoassay (RIA) in the 1950s. After RIA, in the 1960s, the enzyme immunoassay (EIA) was launched, bringing with it considerable improvements such as the substitution of radioisotopes with enzymes, quicker reaction times, better specificities, and a longer shelf life compared to RIA [18,22]. In the 1970s, LFA was still progressing towards greater advancements in the field of diagnosis and development of one POC device, the blood glucose monitor (for diabetic patients). This POC device employs a disposable strip comprising electrodes on which an enzyme is immobilized (dehydrogenase/glucose oxidase enzymes), which significantly helps in the detection of glucose levels in blood [23]. After the 1970s, LFA continued to be refined through the early 1980s, with the filing of many patents on this method via many companies such as Becton Dickinson & Co., Unilever, and Carter Wallace. After the 1980s, LFA was also firmly established during the 1990s and 2000s up until now, and many patents have been filed on numerous features of this technique. Lateral flow tests were initially made available commercially in 1984 as an easy, at-home, urine-based

pregnancy test [24]. However, amongst all the commercialized applications, the human pregnancy kit is the most popular one due to its rapid testing, and it has been given repeated historic attention regarding urine testing for medical diagnostic resolutions. The COVID-19 pandemic has brought LFA into the spotlight once more in recent years (2019–2023), and LFA showed that it could be employed during the lockdown and quarantine phase without involving the medical and healthcare sectors. Despite so much improvement in LFA, scientists continue to strive to improve the kit with unique features that benefit public health; for this effort, they have been recognized by governments. In 2020, Dr. Navin Khanna received India's fourth-highest civilian award, the Padma Shri, for developing diagnostic kits for the detection of the dengue and chikungunya viruses [25]. Among his contributions is the detection of hepatitis B virus surface antigen using upconverting nanoparticle reporter–based highly sensitive rapid lateral flow immunoassay [26]. In this regard, authors have developed a lateral flow immunoassay (LFIA) for HBsAg to provide high-sensitivity rapid diagnostic tests (RDTs) since available RDTs possess inadequate sensitivity and are therefore not suitable for diagnosis of patients with low levels of HBsAg and for blood screening. A reader apparatus can read the UCNP-LFIA results in 30 min, utilizing whole blood, serum, or plasma as the test sample. The designed UCNP-LFIA has a Limit of Detection (LoD) of 0.1 IU HBsAg/mL in spiked serum compared to a commercial conventional optically read LFIA, whereas the LoD of the conventional LFIA was 3.2 IU HBsAg/mL. The proposed UCNP-LFIA meets the WHO blood-screening requirement (LoD 0.13 IU HBsAg/mL) in terms of LoD. The UCNP-LFIA and traditional LFIA were compared using sample panels with certain characteristics. In the HbsAg Performance Panel, the UCNP-LFIA recognized 20/24 HbsAg-positive samples and 8/10 samples in the Mixed Titer Performance Panel, while the traditional LFIA only detected 8/24 and 4/10 samples in these panels, respectively.

Another contribution by Dr. Khanna includes detection of Anti-HIV-1 and -2 antibodies [27]. In this regard, authors developed an LFA with UCNPs for anti-HIV-1/2 antibodies, and the effectiveness of the assay was thoroughly assessed using complicated patient sample panels. The UCNP-sensitivity of the LFA and its specificity were 96.6% and 98.7%, respectively. For a comparison between the UCNP-LFA and a traditional visual RDT, a different set of samples was used. In this analysis, the UCNP-LFA and conventional RDT possessed sensitivities for HIV-1 of 96.4% and 97.6%, respectively. For both assays, the specificity was 100 percent. The constructed UCNP-LFA indicates usefulness of UCNPs for detecting anti-HIV antibodies. A reader device is employed for measuring the signal, which may enable automated result interpretation, data archiving, and transmission from decentralized sites.

1.2. Components of LFA

LFA involves various components such as a sample pad, a conjugate pad, a nitrocellulose membrane, and an adsorbent pad. To construct the LFA, the first component used is a sample pad. This pad is made up of glass fiber or cellulose. In this pad, the process begins by applying the sample on the pad itself. Its purpose is to transport the sample to other parts of the LFA strips. The sample pad should be able to carry the sample in a smooth, constant, and homogeneous fashion. Sample application pads are occasionally used to pre-treat samples before transporting them. This pre-treatment may comprise sample component separation, interference elimination, monitoring pH, and so on. The second component after the sample pad is a conjugate pad. This pad is made up of polyesters, glass fibers, cellulose, and much more. Biorecognition molecules are located in the conjugate pad that has been labelled and dispensed. The substance of the conjugate pad should instantly release the labelled conjugate upon contact with a moving liquid sample. The labelled conjugate should hold steady for the duration of the lateral flow strip. The outcomes of the experiment can be considerably changed by changes in the dispensing, drying, or release of the conjugate. Inadequately labelled conjugate preparation can reduce test sensitivity. After the conjugate pad, next comes the nitrocellulose membrane. This membrane comes

in a variety of grades as per the construction of nitrocellulose membranes. The flow of the sample covers a distance of 4 cm/s on a membrane, and this membrane has test lines with different widths due to the pore size of the membrane. If the pore size is large, then the flow rate takes a long time to analyze. Thus, for constructing the LFA, different widths and different sizes should be used and optimized because these sizes affect the flow rate as well as the intensity of the signal upon the membrane, and this membrane is responsible for the detection of the desired target [28]. This membrane consists of two lines, i.e., test and control lines. A good membrane should offer both stability and excellent binding to capture probes such as aptamers, antibodies, and others. Nonspecific adsorption over-test and control lines can greatly alter assay findings; hence, a good membrane will have less non-specific adsorption in test and control-line areas. The nitrocellulose membrane's wicking rate can affect test sensitivity. The last component of the strips is an absorbent pad, at the end of the strip, which serves as a sink. It also aids in controlling the flow velocity of the liquid through the membrane and prevents sample backflow. The ability of an absorbent to retain liquid can have a significant impact on test findings. All these pads and membranes are mounted and assembled upon a backing card. The cassette, which houses and shields the testing strip from harm, is the final part of the LFIA [29]. A flexible plastic cover provides support and a platform for the delicate paper strips. It does not have any role in the detection of the target but is present to provide a sophisticated appearance and also ease the handling of the strip as well as control the liquid flow [9]. Assembling components on single strips leads to the development of LFA and can be used for the identification of various analytes.

1.3. Principle of LFA

The basic idea behind LFA is quite straightforward and easy to understand. First, the sample (consisting of an analyte) is put onto the sample pad, ensuring that the analyte it contains can bind to the conjugate capture reagents and the membrane. The treated sample moves through the conjugate pad, which contains antibodies that are specific to the target analyte and have been coupled to mostly gold nanoparticles (fluorescent particles), before migrating into the detecting zone together with the conjugated antibody. Nitrocellulose membrane, a porous membrane commonly constructed of nitrocellulose containing immobilized biological components, is the term used to refer to this detecting zone (mainly antibodies or antigens). The nitrocellulose membrane's purpose is to attach to the conjugated antibody and engage in analyte-antigen interactions [30]. During the analysis, it serves as a platform for both reactions. Strong nitrate ester dipoles and peptide bonds in antibodies interact to form an electrostatic interaction and is then dried at room temperature after interacting with the test membrane to permanently attach the antibodies to the nitrocellulose membrane. Other compounds, such as polyvinyl alcohol, prevent free active sites on the membrane. The sucrose solution aids in the free mobility of the tagged antibody. When an aqueous sample is poured, it generates a hydrated glazing that dissolves readily and quickly. Capture molecules, also called bio-recognition elements (nucleic acids and antibodies), are coated on the nitrocellulose membrane through electrostatic interactions and hydrophobic forces/hydrogen bonds to build test and control lines [28]. Since the membrane has two different types of lines (test and control lines), a reaction on the test line demonstrates the recognition of the sample analyte, while a response on the control line checks proper liquid flow over the strip. Following the incubation process, a single line on the pad specifies the positive and negative outcomes of this test. The read-out of this test, denoted by these lines with varying intensities, can be examined visually or via a specialized reader. Finally, the remaining sample is able to complete the sample flow when it reaches the absorbent pad, which has a large bed volume [31].

1.4. Manufacturing of LFA

Concerning engineering and manufacturing, the high flexibility of LFA is provided by the easy lamination method used to combine diverse membrane components. To

manufacture an LFA, only a few instruments are involved, such as a Dispenser, Membrane cutter, and Assembly roller [16]. The construction of an LFA-based biosensor begins with a laminated card that serves as a base for the strips, and then all of the membranes and various pads (Nitrocellulose membrane, sample pad, conjugation pad, and adsorbent pad) are placed on top of it. On the nitrocellulose membrane, two lines, one test line and one control line, are drawn and dried. The gold-nanoparticle-bio-recognition complex sample is deposited on the conjugation pad using a spray machine. Finally, the strips are trimmed and inserted within the plastic cover, and the LFA is ready for testing (Figure 1).

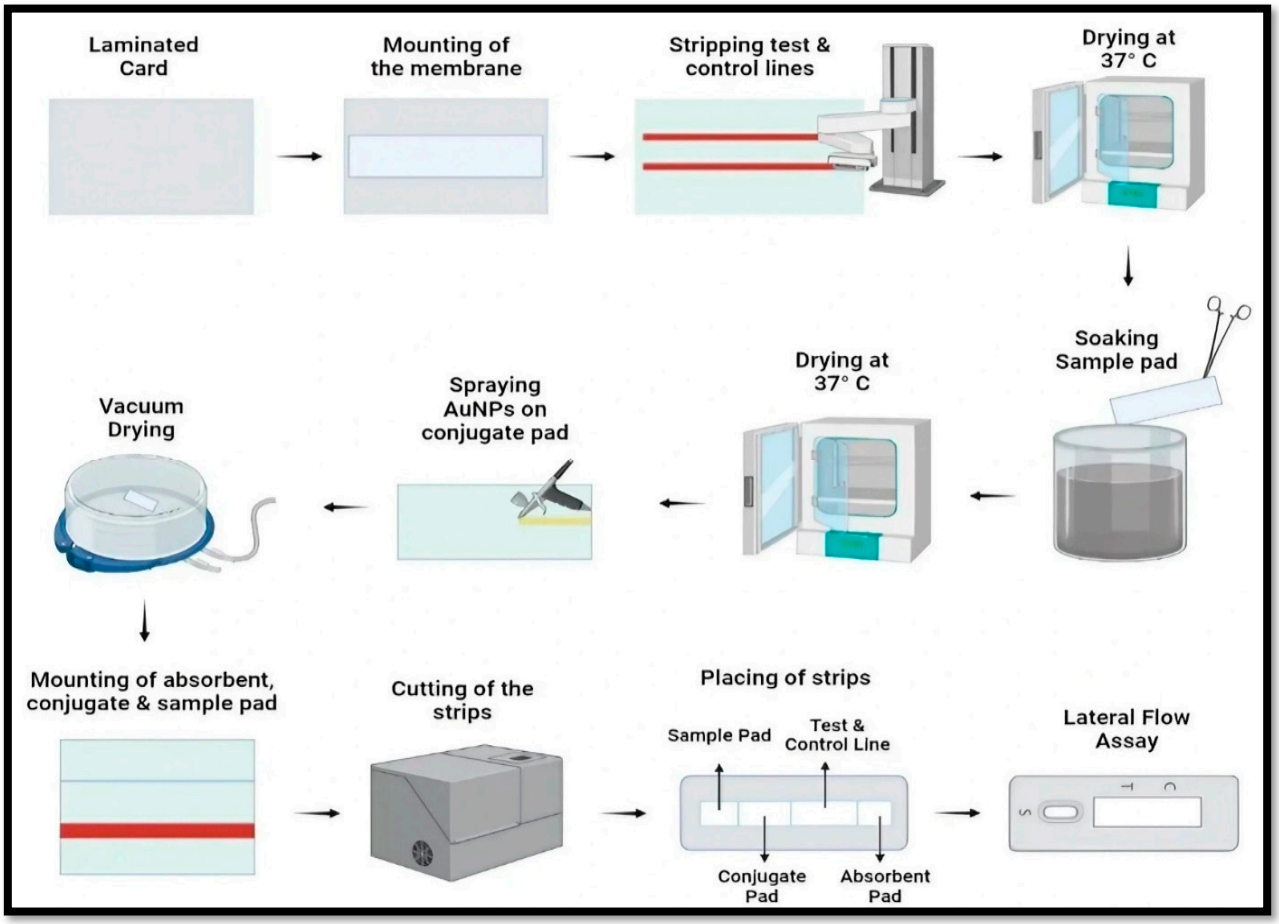

Figure 1. Step-by-step protocol of LFA engineering. Beginning with a laminated card, Mounting of membranes; stripping test and control line settings; Drying at thirty degree Celsius; vacuum drying; Spraying of gold nano-particles on conjugate pad; Drying again; Soaking sample pad; Mounting of absorbent, conjugate, and sample pads; strip cutting; strip placing inside plastic cover; LFA ready (adopted) [15].

The step-by-step protocol of LFA manufacturing is briefly explained in Figure 1.

1.5. Advantages and Disadvantages of Lateral-Flow-Assay-Based Biosensors

Every technology possesses advantages and disadvantages, which is why LFA meets both aspects of usability. Still, LFA possesses numerous advantages over other biosensors, including low cost, quick response, naked-eye detection, requiring a small amount of sample, and portability. All of these advantages make this approach more appealing when compared to other techniques, but LFA also has several disadvantages that should be addressed for the benefit of users, such as response time varying with different pores sizes; holes being blocked by matrix components; if the sample is dropped onto the sample pad, it is impossible to stop the testing; and it is a very dedicated assay that requires

proper handling with a focused mind. Table 1 summarizes the list of advantages and disadvantages associated with an LFA-based biosensor.

Table 1. Advantages and disadvantages of LFA (adopted) [9,30].

S.NO.	Advantages	Disadvantages
1.	- Rapid, inexpensive, and low-sample volume - One-step test, no need for washing stages - Less analysis time	- Mostly qualitative or semi-quantitative. - One-step assay
2.	Ease of device preparation	From one batch to the next, reproducibility varies
3.	Extremely long shelf life and environmental stability across a wide range	Most of the devices are capable of simultaneously detecting more than one or two analytes
4.	- Effortless and user-friendly process - Very little energy consumption	Poor affinity of biomolecules for analytes and a tendency for cross-reactivity. - No way to increase the response through an enzyme reaction. - A good antibody preparation is essential.
5.	Requirement of small sample volume	- Pre-treatment of the sample is occasionally necessary and takes time. - For samples other than fluids, sample preparation is required. - Precision is decreased by inaccurate sample volume - Holes can be blocked by matrix components
6.	Most of the time allows sample application without pretreatment	- It is impossible to slow down or speed up capillary activity once the sample has been placed to the strip. - Limiting the total volume of the test sets the sensitivity.
7.	- Variety of applications - Format, biorecognition molecule, label, and detecting system versatility - Electronics integration is simple - Comparable to or superior to other well-established approaches in terms of sensitivity and specificity. - High commercialization potential	- Analysis time is influenced by the characteristics of the sample, such as viscosity and surface tension. - Very delicate assay

2. Different Formats of LFA

2.1. Sandwich Format & Competitive Format

Depending on the type of target analyte, the LFA platform offers a variety of stream formats. The sandwich test and competitive assay are the two most popular types. With analyte antigens that bind to two separate types of antibodies and have two distinct epitopes, a sandwich test is frequently utilized. Since the targeted antigen is entrapped amidst the two antibodies at the test site, the signal strength is proportional to the analyte concentration in the sample [32]. The competitive test, on the other hand, is frequently employed when the target is a tiny molecule, such as a pesticide, veterinary medicine, mycotoxin, or heavy metal, which has only a single antigenic determinant and cannot bind two antibodies at once. Additionally, contrary to the sandwich format, the outcome is positive when the target biomolecule is present and the signal intensity on the test line drops/disappears [33].

2.2. Antibody and Aptamer-Based Formats

LFA can be created using a variety of recognition elements including antibodies, aptamers, and molecular beacons. Despite the fact that, in LFA, antibodies are the foremost utilized biorecognition component, the antibody-based platform used to detect a wide range of targets is always constrained by production time, stability, and effects of antibody alteration. Finding alternatives to antibodies is a very important and popular topic of

research. Typically, numerous lysine residues (through primary amine) are used to post-modify antibodies, and the diverse distribution of labels might make it difficult to construct quantitative assays, leading to some ambiguity, resulting in antibody deterioration [34]. LFA based on antibodies is referred to as LFIA, i.e., lateral flow immunoassay [35]. Nucleic acid-based diagnostic assays have received great interest recently. Aptamers are scaffolds made of nucleic acids that have the capacity to bind to a wide array of target molecules. Aptamers are synthetic, short, single-stranded DNA or RNA molecules with molecular weights between 10 and 30 kDa. DNA is frequently used since it is more stable than RNA and facilitates the selection of aptamers [36–38]. Due to their distinctive three-dimensional form, aptamers can bind to their target analytes through a variety of binding mechanisms, including Van der Waals forces, hydrogen bonds, aromatic ring stacking, salt bridges, various electrostatic interactions, and shape complementarity. Aptamers are known as "chemical antibodies" due to their chemical properties, implying that they act similarly to protein antibodies. Due to their strong affinity and target-specificity, aptamers can effectively compete with antibodies. A good replacement for antibodies when creating LFAs can be aptamers due to their inherent advantages. Aptamer-based LFAs present novel possibilities for creating cutting-edge diagnostic platforms. Aptamers, for example, can hybridize with complementary DNAs and then disassemble hybridization when they come into contact with a target molecule. Additionally, aptamers in some detection systems can be renewed through heating or other processes. Aptamers are appealing for creating low-cost, reusable, and robust analytical instruments, notably for LFA, because of these benefits [39,40].

2.3. Multiplex Format and Nanoparticle-Based Formats

Given its obvious potential to save time and samples, multiplexing—the simultaneous detection of different analytes in a single sample—has been studied for many years. Multiplexing reduces sample volume, time, and cost because the majority of diagnostic samples are extracted in small amounts (such as blood and nasopharyngeal swabs) [41]. There are a few common approaches for multiplexed LFAs: (a) testing for various targets on various test lines or array places on a single test strip; (b) multiple colors or multi-fluorescence signals (from dyes, quantum dots, and up-converting phosphor) on a single test strip, allowing for the recognition of various targets on a single test line and the differentiation of those targets by a single color or combination of colors (additionally, multiple test lines may be present with each labeled a different color or fluorescence); and (c) using several channels that resemble microfluidic devices or 2D patterning to test for various targets on each channel. Each channel may additionally include multiple test lines or array locations to further boost throughput. In some situations, and commercial goods, several parallel test strips are combined in a single device cassette for the parallel running of samples, each to detect a separate target [42]. In addition to the simultaneous detection of numerous analytes with parallel usage of them or single-target detection in a single strip, multiplex LFAs (mLFAs), based on the use of multiple dots or TLs in a single strip, have recently appeared. As a result, the employment of several tags makes it possible to distinguish between them and quantify them, saving both time and money on the assay while also reducing the volume of material needed. To date, a wide range of tags have been used, from recently developed diversified tags for quantitative tests to polystyrene micro-beads and GNPs as colorful tags for early qualitative assays [43]. In the creation of a lateral flow strip, the use of nanoparticles (NPs) as labels is crucial (LFS). The effectiveness of these devices is directly impacted by the NPs selected and the associated detecting technique. Various nanomaterials (including, but not limited to, latex beads, quantum dots, carbon nanotubes, and gold nanoparticles) are used in LFBs. One common NP is AuNP, which is easy to make and manipulate, stable over time, size-tunable, biocompatible, and has a strong red color that can be seen even with the naked eye. Color readers are typically used to improve detection limits. These characteristics make AuNPs the most frequently mentioned nanomaterial utilized as an optical label in LFSs [42,44].

2.4. CRISPER-Based Format

The most trending technique in the field of diagnosis is CRISPR. Numerous applications of CRISPR and CRISPR-related (Cas) genes led to their selection for the 2020 Nobel Prize in Chemistry [45,46]. A diagnostic tool based on CRISPR can detect viral nucleic acids at attomolar concentrations. Compared to RT-PCR-based methods, which can only detect femtomolar quantities, they are more sensitive. Fast and accurate detection of nucleic acids isolated from these various samples is made possible by LF CRISPR-based biosensing. Researchers constructed an LFA strip and integrated this method with the CRISPR-Cas system in a study published in 2020 to make it affordable for on-site diagnosis. Researchers found that Cas-LFA may achieve a rather low detection limit with 100% specificity in less than an hour, suggesting that it is a promising choice for upcoming Point-of-care testing. However, additional study is required to decrease the assay time and CRISPR-Cas identification stage [32].

3. Common Applications of LFA

There are various common analytes that are easily detected by just one single strip, i.e., LFA and its quite popular and essential worldwide. COVID-19 rapid antigen kit, pregnancy test, diabetes test, Alzheimer's test, and dengue test are some of the examples.

3.1. COVID-19 (Coronavirus)

Recently, the world witnessed the deadly outbreak of the infectious virus COVID-19. Proper diagnosis was the only survival effort to stop this at the initial stage. Many scientists developed cheap and effective LFA kits for the detection of this virus. Recently new methods have been utilized and integrated with lateral flow assay to upgrade the platform towards LFA-based Covid diagnostics. For example, Osborn et al. [47] utilized a trending method called CRISPR/Cas9, which is a protein that can be easily manipulated towards the specific binding with the sequences of DNA/RNA and helps in the effective detection of the targets. In this work, they integrated an LFA with CRISPR/Cas-9 for the detection of SARS-CoV-2 (ORF8a gene sequence). Usually, Cas proteins' nuclease characteristics are used to create fluorescent signals when they come into contact with targets. Based on these ideas, they created a sensitive fluorescence-based test for detecting the SARS-Cov-2 sequence. This idea depended on a DNA probe containing a quenched fluorophore binding to the target and being cleaved to Cas9. Other than SARS-CoV-2, the proposed LFA is also able to detect influenza A/B and RS-virus (respiratory syncytial). The multiplexing fluorescence-based detection capability of this lateral flow assay is effective for field-based diagnostics. The ability to identify two genes in a single test, as employed by the gold standard RT-qPCR method, is a vital parameter to boost the accuracy rate and is currently a limitation facing a few methods for identifying SARS-CoV-2. Another study by Xiong et al. [48] developed an assay for swift and concurrent dual-gene testing of SARS-CoV-2 on a single platform of an LFA strip using CRISPR/Cas9-mediated triple-line lateral flow assay (TL-LFA) and multiplex reverse transcription-recombinase polymerase amplification (RT-RPA). This assay, which has a sensitivity of one hundred RNA copies/reaction (25 μL), is described for the detection of the envelope (E) and open reading frame 1ab (Orf1ab) genes from cell-cultured SARS-CoV-2 and SARS-CoV-2 viral RNA standards. In addition, dual-gene evaluation of sixty-four nasopharyngeal swab samples revealed 100 percent poor prognostic compatibility and 97.14 percent good predictive compatibility. In low-resource areas, this assay provides a more precise and accessible method for diagnosing coronavirus as well as other viral disorders. Kim et al. [49] also coupled the LFA with a new method, i.e., the nanoelectrokinetic (NEK) method. This unique combination of both the approaches (microfluidic paper- based NEK and LFA) significantly improved the LOD as well as sensitivity. In this work, a SARS-CoV-2 IgG sample was preconcentrated from a sample of serums using NEK and assisted with LFA for self-testing. The LFA's LOD increased 32-fold after preconcentration, with an increase in quantitative sensitivities (16.4%), which may present a new option for point-of-care testing and self-testing. In another study,

temperature-based diagnosis was also one of the methods used for effective diagnosis. Temperature sensing is a possible approach for improving the lateral flow immunoassay (LFIA) detecting sensitivity for POC diagnostics. Photoexcitation of reporters such as GNPs and colored latex beads (CLBs) upon the strips of lateral flow immunoassay with a laser power less than 100 mW may easily obtain a temperature rise of more than 100 °C. The mechanisms underlying photothermal detection, despite their potential, are still poorly known; hence, Azuma et al. [50] provided an insightful understanding of this thermometric experiment using non-fluorescent CLBs deposited on the membrane (nitrocellulose) as the reporters. In this study, they tested the effect of temperature rises on the number density of membrane-bound colored latex beads and found that red (and black) latex beads absorbed light 1.3 and 3.2 times more efficiently at 520 nm. The improvement was related to multiple light scattering in this very porous media, a process that might have a major influence on the enhancement of LFIA sensitivity. The detection limit was 1×10^5 particles/mm^2. Compared with prior research that used GNPs as reporters, this CLB-based thermometric test has a tenfold greater sensitivity than color visualization. This study demonstrated the experimental usage of the thermometric immunoassay using COVID-19 rapid antigen assays. Another team of researchers, Dighe et al. [51], detected COVID-19 with the help of cysteamine capped-gold-NP-based lateral flow assay with 99.99% accuracy and specificity in a short period of time (30 min) and also compared the results with the RT-PCR kit. In this experiment, they utilized 6-FAM and biotin-labelled antisense oligo-nucleotides (probe) to target the N-gene sequence of SAR-COV-2. This is an RNA extraction and amplification free nucleic acid test, which makes this test more promising for point-of-care demand. In another study, Park et al. [52] presented a unique lateral flow assay based on the instrument-free approach of salt-mediated immobilization of nucleic acids. This assay improved the time period, color intensity, and long-term stability, which can be beneficial for many targets such as SARS-CoV-2.

3.2. Diabetes

Diabetes mellitus is one of the leading diseases around the globe and is also considered a rapidly growing disease. To control this, many commercial biosensors have been developed to diagnose this disease and monitor the progression of diabetes' different biomarkers, including glycated-hemoglobin/albumin and 1-5-anhydroglucitol. Mostly electrochemical biosensors have been developed by researchers; however, recently, many scientists have been focusing on low-cost POC and paper-based biosensors. Ang et al. [53] developed an LFIA based on colloidal gold utilizing a sandwich format of LFA that can detect type 2 diabetes mellitus biomarker (HbA1c). The proposed immunosensor is preferable compared to an electrochemical-based biosensor in terms of its low price and point-of-care detection of HbA1c. Other than HbA1c, there are numerous biomarkers that can also be used to detect diabetes such as vaspin, which was utilized by Ali et al. [54] for the confirmation of diabetes (type-2) in a sample with the help of LFA based on fluorescent upconverting NPs. In their method, an aptasensor sandwich pattern was applied for the detection of vaspin with a limit of detection of 39 pg ML^{-1}, which proved that the LFA can confirm diabetes by detecting vaspin levels because its actual range in human blood ranges from 0.1 to 7 ng mL^{-1}. In another study, Ki H et al. [55] developed a paper-based biosensor, i.e., LFA for the detection of glucose and glycation levels of human serum albumin with R-squared values of 0.932 and 0.930, respectively, while the average determination recoveries of both the ratios to monitor diabetes mellitus were 98.32 percent (glycation) and 85.80 percent (glucose). Thus, this platform can easily determine the different ratios of diabetes simultaneously. Recently, Belsare et al. [56] developed two LFAs for the detection of two target GAs (glycated albumin and total serum albumin) and coupled these LFAs with a handheld reader that helped in reading the signals, which significantly showed good ranges of the GAs (3 to 20 mg mL^{-1}) and serum albumin assay (20 to 50 mg mL^{-1}) without utilizing any sample dilutions. Both developed lateral flow assays were subsequently combined into a single dual assay cartridge, allowing both assays to run concurrently and

deliver the percentage of glycated albumin value in a single test. As a result, the dual assay cartridge with the reader system has the potential to provide an efficient platform for measuring GA for gestational diabetes mellitus tracking at the point of care.

3.3. Pregnancy

One of the oldest LFAs was developed for the confirmation of pregnancy status in women. Pregnancy in women is based on levels of a hormone: the human chorionic gonadotropin hormone. The level of HCG increases after fertilization and can be detected in urine after seven to ten days. The working principle to detect the pregnancy status is very easy. Urine is moved via sample and conjugate pads when it is dropped on the strips. In this stage, a label-analyte complex is formed if hCG is detected in the urine because it will bind to the color particles that have been conjugated with hCG antibodies. The substance passes through the test line along with the urine, where it is detected using a sandwich assay technique by another hCG antibody that is present in the line. It aggregates when the complex is caught, producing the signal at the testing line showing that the woman is pregnant. Because a pregnant woman's HCG expression is noticeably higher than that of a non-pregnant woman's, the test can easily discriminate between the two [57]. As the working principle of the pregnancy kit is very easy and simple due to its non-invasive approach, it makes this assay more reliable for pregnancy testing compared to other targets such as blood-based testing. However, many scientists try to modify this pregnancy assay for high specificity and sensitivity, and have created more advanced tests in terms of digital platforms. For example, Zhang et al. [58] detected HCG via a portable test strip of LFA integrated with a smartphone, which helped in optimizing an image algorithm in the context of HCG detection with LOD of 3 ng/mL and possessed a good range (6–300 ng/mL). In the test, a colloidal gold HCG sensing strip depending on the antigen-antibody immune response was built, and the study findings of three different image analysis techniques upon the same strip identification were compared, with results showing that the last algorithm could achieve the best recognition of the region of strip interest. In another study, Danthanarayana et al. [59] developed a multiplex-based LFA for the detection of two targets (HCG and prostate-specific antigen) on a single platform and integrated it with a smartphone-based imaging system that successfully aided in analyzing the target detection with a LOD of 1 ng mL^{-1} (HCG) 0.1 ng mL^{-1} (PSA). In this work, they used two persistent luminescent nanophosphors (green and blue emitting), and both of these were responsible for emitting multiple colors, which helped in identifying the multi-analyte on a single strip on the basis of color. Another work by Yan W et al. [60] developed a labeled lateral flow immunoassay utilizing MNPs, i.e., magnetic nanoparticles, coupled with a machine learning model that helped enhance sensitivity and specificity as the model categorized the weakly positive and negative samples. The developed platform potential was evaluated using two samples, i.e., HCG and myocardial infraction serum samples. The range of HCG was 1–1000 mIU ML^{-1}. The cardiac markers also showed good linear correlations with standard values. This study revealed that different biomolecules can also be detected on a single platform, and thus this multiplex platform is also applicable for other applications such as food testing, environmental testing, and national security.

3.4. Alzheimer's Disease

LFAs also show promising results for neurological illnesses. Alzheimer's disease is intimately linked to neurodegenerative disorders. Several researchers, including Brazaca et al. [6], developed LFA assays for the detection of Alzheimer's disease blood biomarkers (fetuin B and clusterin), and their findings were examined using image analyzers. They employed gold nanoparticles, which had high sensitivity and linearity, with R^2 values of 0.988 and 0.998 for fetuin B and clusterin, respectively. Using such blood indicators for Alzheimer's disease undoubtedly aids in the early detection of the disease. Recently, Zhang et al. [61] (2023) developed a lateral flow test for the detection of an Alzheimer's disease biomarker (plasma phosphorylated tau) for early-stage diagnosis of the disease.

They integrated LFA with colorimetric and Surface Enhanced Raman Scattering for dual-readout LFA for the efficient detection of AD biomarkers, yielding good findings with a LOD of 60 pg/mL through the naked eye and 3.8 pg/mL with SERS and no cross-reactivity with other species.

3.5. Dengue Virus

Dengue is an infectious virus-based illness spread by female mosquitos. Several researchers developed LFA for the detection of the dengue virus. Martinez et al. [62] Established a gold nanoparticle-based LFA for the detection of the dengue virus, which successfully identifies the target at a LOD of 5.12×10^2 PFU. In another investigation, Le et al. [63] Developed an LFA-based immunosensor capable of detecting all four NS-1 serotypes. In their study, they used fluorescent nanodiamond as a reporter, which demonstrated a superior response compared to traditional label tags such as gold NPs. They combined this LFA with spin-enhanced approaches for improved results, demonstrating a wide determination range of dengue NS-1 serotype concentrations ranging from 0.1 to 1.3 ng/mL. Recently, Lu et al. [64] (2023) created an LFA based on aptamer for the detection of the dengue virus with an LOD of 24 pg/mL, and their findings could be observed with the naked eye. Thus, these are the few findings accessible on the dengue-virus-based LFA biosensor, and such results verify that infectious diseases may be readily determined using the simple LFA technique with outstanding sensitivity and selectivity with low-budget-based dengue-virus testing.

4. Advanced and Futuristic Applications

Although LFA is one of the simplest techniques to detect various diseases, nowadays, scientists try to advance this technique beyond imagination and improve these biosensors to the next generation, which aids this technique in every aspect of health management. Table 2 summarizes all the applications based on LFA.

4.1. Robotic-Based LFA Application

Currently, robotic science is truly a boon to every industry due to its versatility, and it can be used in every field and can even become a potential candidate in the diagnostic field (Figure 2). Very few researchers have utilized robotic science in the development of LFA, excluding Huynh et al. [65], who developed a lateral flow assay and integrated it with a robotic system used for designing experiment software and robotic liquid-handling hardware. Using these robotic system helps in various aspects, including reducing the labor burden, increasing the size of experiments, speeding up the testing development process, facilitating larger experiments, and eliminating the need for extra laboratory experts in experimental laboratories for interpreting outcomes. In their work, the system's ability to conduct trials with both discrete and continuous variables—covering the vast majority of assay development research—was established. While this approach has been used to produce malaria antigen lateral flow assays, it may also be used to develop other lateral flow assays and test types. This method might help with diagnostic development in resource-constrained environments since it enables more systematic and quick assay development. Another team of researchers, Anderson et al. [66], developed a rapid lateral flow assay and coupled it with an automated liquid-handling robot. Large-scale optimization trials with discrete and continuous variables, such as antibody pair selection and reagent concentration, are possible with the use of the program's lateral flow assay-specific hardware and software. The platform's first validation was proven during the production of a malaria LFA, but it was easily upgraded to include the construction of SARS-CoV-2 and Mycobacterium TB-lateral flow assays. A direct comparison between the robotic platform and a more conventional ELISA-based approach served to assess the platform's validity, which executes optimization trials directly upon lateral flow tests rather than in solution.

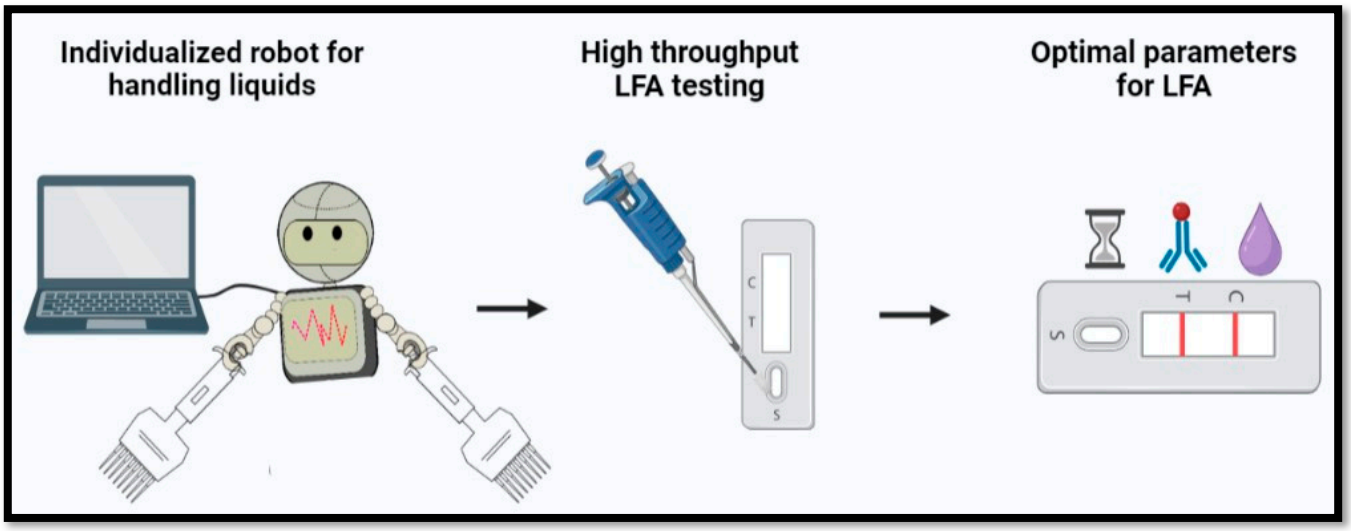

Figure 2. Diagrammatic representation of robotic-based LFA: Individualized Robot for handling liquids; High throughput Lateral Flow Assay Testing; Optimal parameters for Lateral Flow Assay, i.e., different time response, selections of bioreceptors selections, and targeting of different bodily fluids.

4.2. Cotton-Thread-Based LFA

Despite being integrated with the LFA technique, which is an expensive and a sophisticated platform, LFA can be easily coupled with cheap textile materials such as cotton thread to make LFA more profitable in terms of its low-cost and sensitivity diagnosis, and such methods are referred to as thread-based microfluidic detection approaches. Such approaches are gaining popularity because they offer various benefits over other materials. Due to capillary action, threads, like paper, may spontaneously transport fluid, though they are stronger mechanically and do not require hydrophobic barriers. As a result, thread-based microfluidic devices may be made cheaply without the use of external pumps or costly microfabrication equipment. Despite these exceptional properties, obtaining a regulated and constant flow rate remains a difficult operation, owing mostly to fluid evaporation [67]. Because of its easy availability, low weight, support for transporting and mixing liquids, strong capillary force, cheap cost, and outstanding biocompatibility, cotton threads with spiral structures constructed of cotton fibers have been employed as a novel form of the microfluidic channel in point-of-care devices and can be easily integrated with lateral flow assay. Shen's team created thread-based and thread-paper-based 3D-structure lateral flow biosensors for nitrite ions and uric acid, two crucial indicators. They showed that thread is a good material for making lateral flow devices. Additionally, Whitesides' team created three cotton thread-based assays: a "woven array," "branching pattern," and a "sewn array." The thread-based devices are capable of detecting five distinct analytes. Thread was weaved on a loom to allow numerous tests to run concurrently or was sewed through a hydrophobic polymer sheet to insert assays into bandage-like devices, demonstrating its capacity to run many assays concurrently [68]. Another study by Zhang et al. [69] developed a sensitive LFA and integrated it with cotton thread. In order to slow sample flow and increase reaction time, in their study, cotton-thread-based obstacles were embedded into strips of paper. The cotton threads were implanted, and their hydrophilicity was gradually tuned. A mathematical circuit-like model was used to simulate the liquid flow rate in the cotton-thread-embedded lateral flow test, and the simulation results corroborated physical results. The cotton-thread-embedded lateral flow test revealed a fourfold increase in detection sensitivity over unmodified lateral flow assays using HIV-nucleic acid as a model target. Future paper-based microfluidic device production shows enormous potential thanks to the strategy of embedding cotton threads into paper.

4.3. LFA with Smartphone and AI/5G Technology/Telemedicine/E-Prescription

Smartphones and artificial intelligence (AI) are popularly used by researchers due to their various advantages such as on-site testing, multitasking, high-quality images, data analysis via AI, and can upload data for cloud figuring. Currently, 5G technology is also emerging, which will significantly help the healthcare sector and improve telemedicine. Telemedicine makes it feasible for patients to receive all of their medical care without having to visit a hospital. In the near future, consumers will be able to obtain tests at reasonable costs from drugstores near their homes. They may perform the tests at home in minutes and then upload their results immediately and confidentially to doctors in seconds via video calls, which significantly reduces treatment time and risk of spreading infectious diseases in public. If test results are abnormal, doctors will be able to produce electronic prescriptions. In more serious situations, doctors might request more testing if results indicate that a severe condition, such as cancer, is developing at an early stage [57]. There are various biosensors integrated with smartphones. One unique combination is an LFA based on a smartphone for the effective diagnosis of various diseases. For example, Jung et al. [70] detected food-born bacteria (*E. coli* O157:H7) by developing an LFA based on a smartphone as the naked eye cannot effectively report the presence of a test band. Hence, a smartphone was used as a colorimetric reader and effectively categorized the different concentrations on the basis of different color shades of the targets. The smartphone also recorded the results and the time and date of the testing. In another study, Richardson et al. [71] detected SARS-CoV-2 by constructing an LFA coupled with AI. In their study, they used an AI algorithm that helped in testing in significantly large populations for 5 days, and the outcomes could be automatically transferred to the health sector. The outcomes were more sensitive (86.2%) and specific (94.3%) compared to RT-PCR, and sensitivity was enhanced through AI readings (86.2%) compared to the human eye (71.4%). In another study, Tong et al. [72] also developed an LFIA and combined it with AI-assisted colorimetric polydopamine NPs for the quantification of neutralizing antibodies produced by vaccinations with a LOD of 160 ng/mL. A high degree of consistency with a commercial ELISA kit was also shown by the fact that it successfully identified 50 clinical serum samples. PDA-based LFIA technology revealed more precise measuring capabilities for clinical serum compared to commercial gold nanoparticle-based LFIA. Therefore, it is anticipated that the AI-assisted PDA-based LFIA platform will be very beneficial for the large-scale evaluation of vaccination efficacy and other POC-immunoassays. It possesses sensitive and exact quantification capabilities.

4.4. 3D Printing/Additive Manufacturing/Layer-by-Layer Overlapping of Materials/Rapid Prototyping-Based LFA

Apart from improving the sensitivity and selectivity of LFAs, there are several laboratories that are also trying to advance the structure and design of LFAs to make them more sophisticated and appealing in the market. One of the most innovative manufacturing tools, the 3D printer, emerged with Chuck Hull's patent publication in the year 1986, which pioneered stereo lithography; since then, it has progressed and differentiated with the development of novel printing processes and a plethora of materials [73]. Three-dimensional printing, also known as additive manufacturing, is dependent on the layered manufacturing principle, which involves the layering of materials [74]. Currently, the 3D printing technique is also extended towards diagnostic applications to construct innovative sensors and biosensors as it possesses various advantages, including the ability to manufacture complicated shapes with high precision, quick prototyping, material savings, flexibility in making object alterations, and personalization. It can also help print materials with varying densities and strengths and can fabricate supporting components and optical and electronic parts of diagnostics tools as well [75]. Recently, 3D printing machines have become promising candidates to help researchers design the shapes of devices according to their desires conveniently. For example, Alrashoudi et al. [76] constructed an LFIA using different methods, including additive manufacturing techniques. In their study, they

designed different parts to develop a modified LFA device to help in the effective detection of COVID-19. An antibody dispenser was constructed by Han et al. [77] using 3D printed and commercially available materials for the creation of LFA strips in addition to creating the components of LFA. In their study, they demonstrated how anti-C-reactive protein (CRP) and anti-rabbit antibodies can be consistently dispensed to draw test and control lines for CRP detection. The dispenser can additionally use two dispensing needles, which enables the simultaneous administration of various reagents—essential for the production of LFA strips.

4.5. LFA Integrated with Portable Transducers

One of the intriguing technologies involves the combination of a delicate device with a strong transducer. However, doing so is a very complex innovation that involves the development of a unique and effective diagnostic device. There are only a few reports available on such a unique combination in LFAs. Hamad et al. [78] reported an LFIA coupled with electrochemical-based transducers for rapid-testing HCG detection. In their work, they validated results with the help of two techniques (amperometric and impedimetric) by fixing electrodes on the surface of an LFA strip. Their proposed digital lateral flow immunoassay significantly aided in the detection of HCG.

4.6. Flying LAB/Unmanned Aerial System/In-Flight Test/Lab on Drone Coupled with LFA (Lab-on-Drone)

A marriage between technologies from two fields, diagnostic tools and the engineering sector, begot an unmanned aerial diagnostic vehicle, i.e., a Lab–On-A-Drone, which offers a fast, portable, and easy-to-operate diagnostic platform. These drones are interfaced with a smartphone camera and digital computing power [79,80]. There is no report available on a drone integrated with LFA. Recently, drone-based detection has been investigated by various scientists using other sensing technologies. For example, Dawoud et al. [81] detected chemical warfare agents by adding sensor arrays to drones, making this portable device a more prominent option for remote-detecting applications. In this proposed system (an embedded, miniaturized, drone-based platform), they also compared the results of a miniaturized potentiostat with a highly sophisticated laboratory-based potentiostat. In another study, Priye et al. [82] also used drones for the detection of nucleic acid by employing a drone (consumer-class quadcopter). In this work, they also used a smartphone camera and included a picture analysis app for the effective diagnosis of nucleic acid. Thus, on the basis of the above work, in the future, LFA biosensors might be coupled with drone systems that may become more prominent compared to other biosensors due to their low weights, low prices, safe interactions with other airborne drones, and ease of combining with drones. Coupling LFAs with drones may lead to the development of new futuristic technologies such as unmanned-aerial-system-based LFAs.

4.7. Electronic LFA Strip (e-LFA Strip)/Papertronic-Based LFAs

In the future, LFA can be modernized beyond imagination. Combining paper-based LFA with a self-powered electronic setup can lead to a new futuristic technology known as e-LFA. It is well known that LFA is a delicate biosensor that can be turned into a self-powered electronic device that can also be modified with a metallic covering of LFA rather than using a plastic cassette, which makes it more attractive and powerful. LFA is based on paper, and paper can be easily integrated with electronic setups. Only a few studies in the literature have described the relation of paper with electronics. At the end of the 20th century, low-cost printed microelectronics found in cellulose paper provided the best support even for high-end silicon-based electronics thanks to the interesting peculiarities of roughness, porosity, and opacity that are well-suited to electronics deposited on its surface [83–85] (Figure 3 represents a futuristic device). Thus, on the basis of the above literature, in the future, LFA biosensors can also be coupled with electronic setups that may

become more prominent and may lead to the development of new futuristic technologies such as e-LFA/papertronic-based LFAs.

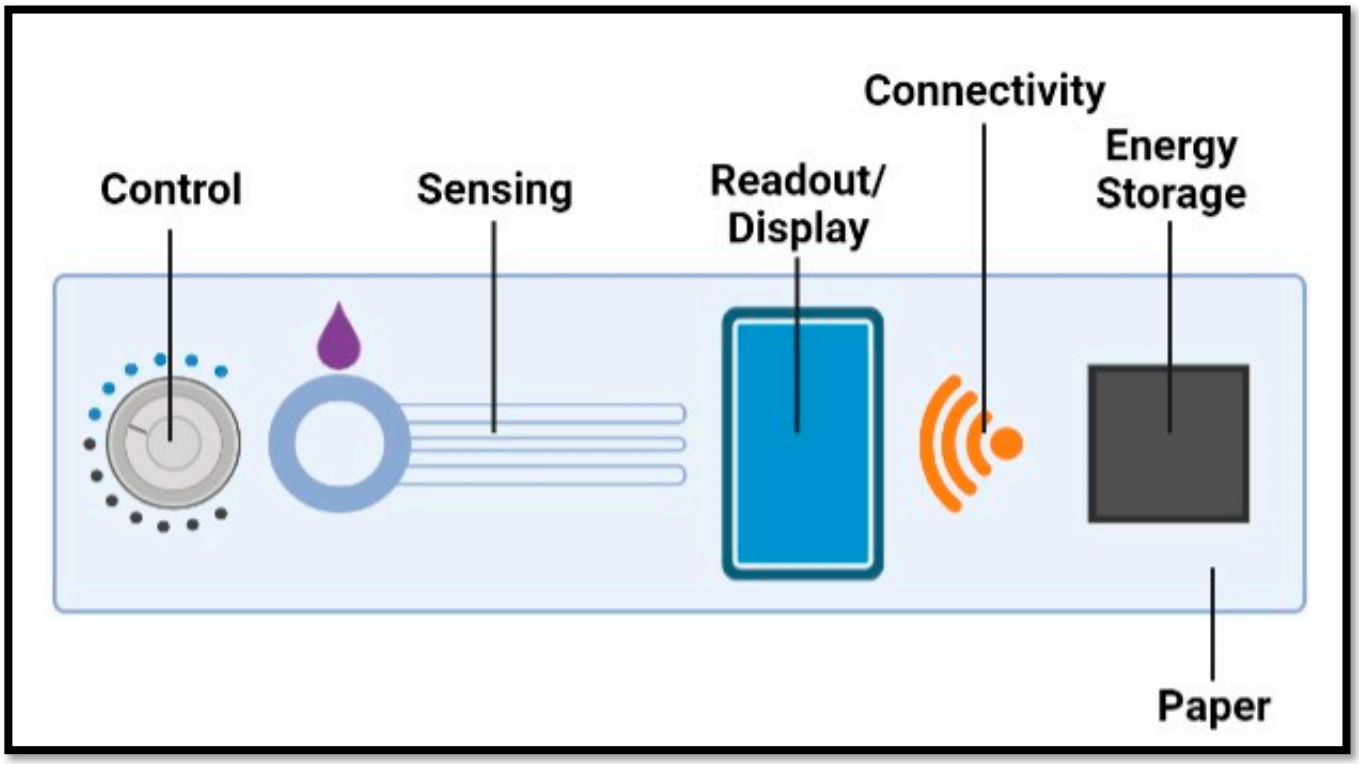

Figure 3. Futuristic e-LFA (electronic Lateral Flow Assay) device that encompasses microfluidics, electronics, and data processing on a single LFA strip.

Table 2. Summary of different applications based on LFA.

Common Applications of LFA					
S.NO.	**Lateral Flow Assay Type**	**Analytes/Diseases**	**Outcomes**	**Label Tag**	**References**
1.	LFA with CRISPR/Cas-9	SARS-CoV-2 (ORF8a gene sequence) and influenza A/B and RS-virus (respiratory syncytial)	-	-	[47]
	CRISPR/Cas9-mediated triple-line lateral flow assay (TL-LFA)	Dual-gene testing of SARS-CoV-2	100-RNA copies/reaction (25 µL)	Gold NPs	[48]
	LFA: nanoelectrokinetic (NEK)	SARS-CoV-2	Increased limit of detection by 32-fold	Orange G color dye	[49]
	Thermometric lateral flow immunoassay (LFIA)	COVID-19	1×10^5 particles/mm^2	Colored latex beads	[50]
	RNA extraction-free LFA	SARS-CoV-2	99.9% accuracy	Cysteamine capped gold NPs	[51]
	Nucleic acid-based LFA	SARS-CoV-2.	300 aM & 500 aM	Gold NPs	[52]

Table 2. *Cont.*

Common Applications of LFA					
S.NO.	**Lateral Flow Assay Type**	**Analytes/Diseases**	**Outcomes**	**Label Tag**	**References**
2.	LFIA: sandwich format	Diabetes type 2 diabetes mellitus biomarker (HbA1c)	4 percent (20 m mol^{-1}) and 12 percent (108 m mol^{-1})	Colloidal gold	[53]
	Aptasensor sandwich-based LFA	Vaspin	39 pg ML^{-1}	Fluorescent upconverting NPs	[54]
	LFA	Glucose and glycation levels	R-squared values of 0.932 & 0.930, respectively	GNPs	[55]
	LFA with a handheld reader	Glycated albumin and total serum albumin	GA (3 to 20 mg mL^{-1}) and serum albumin assay (20 to 50 mg mL^{-1})	GNPs	[56]
3.	Portable test strip of LFA integrated with a smartphone	Pregnancy test: HCG	LOD of 3 ng/mL	Colloidal gold	[58]
	Multiplex-based LFA integrated with a smartphone-based imaging system	HCG and prostate-specific antigen	LOD of 1 ng mL^{-1} and (HCG) 0.1 ng mL^{-1} (PSA).	Persistent luminescent nanophosphors (green and blue emitting)	[59]
	Lateral flow immunoassay with a machine learning model	HCG and myocardial infraction serum samples	HCG is 1–1000 mIU mL^{-1}	MNP, i.e., magnetic nanoparticles	[60]
4.	LFA with image analyzer	Alzheimer's disease blood biomarkers (fetuin B & clusterin)	R^2 values of 0.988 and 0.998, respectively	Gold nanoparticles	[6]
	Integrated LFA with colorimetric and Surface Enhanced Raman Scattering	Alzheimer's disease blood biomarker: plasma phosphorylated tau	LOD of 60 pg/mL through naked eye and 3.8 pg/mL with SERS	NPs	[61]
5.	LFA	Dengue virus	LOD of 5.12×10^2 PFU	Gold nanoparticles	[62]
	LFA-based immunosensor	All four NS-1 serotypes	0.1 to 1.3 ng/mL	Fluorescent nanodiamonds	[63]
	LFA based on aptamers	Dengue virus	LOD of 24 pg/mL	GNPs	[64]
ADVANCED -APPLICATION OF LFA					
1.	Lateral flow assay integrated with a robotic system	Antigen	-	-	[65]
	Rapid lateral flow assay coupled with an automated liquid-handling robot	Malaria antigen	-	Carboxylic blue latex beads or cellulose nanobeads	[66]
2.	LFA integrated with cotton thread	HIV-nucleic acid	0.25 nM	GNPs	[69]

Table 2. *Cont.*

Common Applications of LFA					
S.NO.	**Lateral Flow Assay Type**	**Analytes/Diseases**	**Outcomes**	**Label Tag**	**References**
3.	LFA-based smartphone	Food-born bacteria (*E. coli* O157:H7)	10^4–10^5 CFU/mL	-	[70]
	LFA coupled with AI read	SARS-CoV-2	Sensitivity enhanced with AI read (86.2%) compared to the human eye (71.4%)	GNPs	[71]
	LFIA combined with AI	COVID-19	LOD of 160 ng/mL	Polydopamine NPs	[72]
4.	LFIA with additive manufacturing technique	COVID-19	-	GNPs	[76]
	3D printed and commercially available materials for the creation of LFA strips	C-Reactive Protein	-	GNPs	[77]
5.	LFIA coupled with the electrochemical-based transducers	HCG	-	GNPs	[78]

5. Conclusions and Future Perspectives

The LFA is one of the most popular commercial biosensors available on the market, and it is still a mystery how simple paper can detect dangerous infectious diseases without using any instruments. The reasons behind its popularity are that it is simple to use, it is low cost, provides on-site detection, is portable, and requires no expertise or sophisticated instruments. Therefore, all the credit for quickly bringing this biosensor from the lab to the market belongs to researchers. Recently, scientists have focused on the advancement of this testing for future generations so that it can attract users with its innovative and smart detection capabilities. For the first time, Shu et al. [86] (2023) used cuttlefish-ink-based natural nanoparticles as a multifunctional label tag. In this work, they replaced gold nanoparticles colored tag agents with natural nanoparticles, which significantly detected clenbuterol with an LOD of 0.179 ng mL^{-1}. In another study, Hu et al. (2023) used Bismuth sulphide-based nanoparticles as a signal substrate for the first time in the development of a lateral flow immunoassay for the detection of clenbuterol, with an LOD of 0.1 ng mL^{-1}. Aside from novel colored tag nanoparticle-based agents, the majority of reports are based on antibodies. However, investigators are now using effective bio-recognition elements such as aptamer in place of antibodies because aptamer has several advantages such as its low cost, easy formation, high specificity, and ease of stability. Mao et al. [87] (2023) employed a triple-helix aptamer for the very first time for the construction of an aptamer-based LFA to detect acetamiprid, and also used nanozyme-based nanoparticles, which helped increase sensitivity and broadened the determination range. Li et al. [88], for the very first time, integrated an LFA with SERS (Surface-Enhanced Raman Scattering) for detection of milk allergens, showing an LOD of 0.19 ng/mL for casein and 1.74 pg/mL for α –LA.

These are some of the most recent works based on LFA progress. All of the most recent data on LFA advancements that have been made available are compiled in this review so that researchers working on LFA can access them and use them to improve their LFA devices in order to make them more advanced. Therefore, in the future, LFA can touch the heights of innovations to amaze the world with its effective diagnosis and design.

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