**Lateral flow immunoassays: The future of on-site detection and diagnostics**

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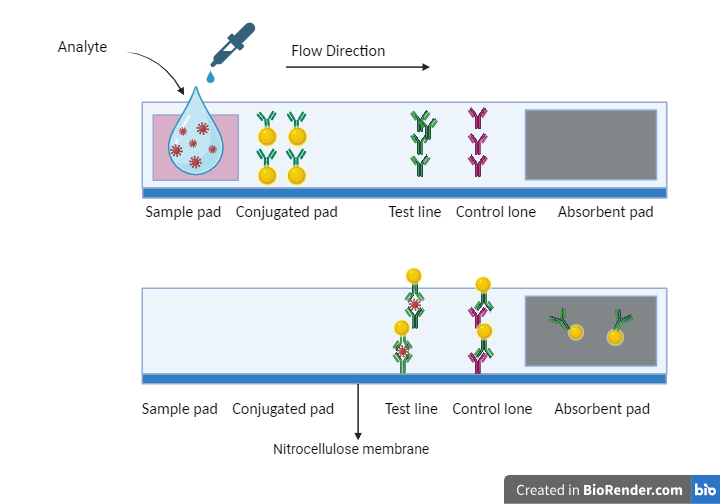
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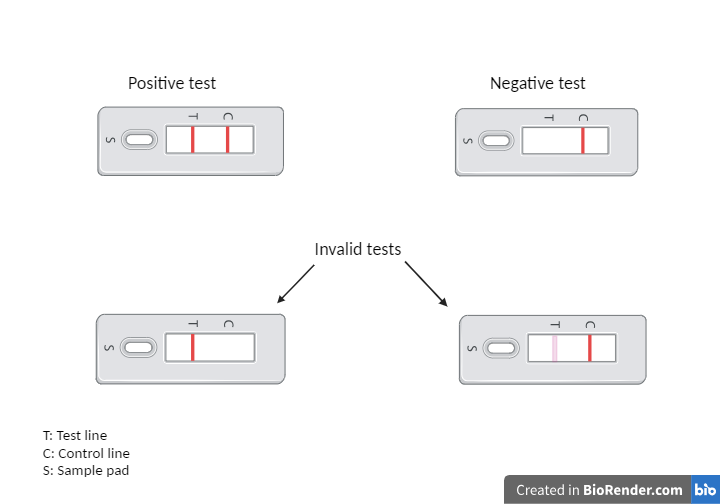
**ABSTRACT**

Lateral flow immunoassays (LFIAs) have emerged as promising future for on-site detection and diagnostics. These assays offer rapid and user-friendly analysis, making them highly suitable for point-of-care testing. The popularity of Lateral Flow Immunoassay (LFA) for point-of-care (POC) diagnostics has grown as a result of its user-friendliness and widespread demand. With rising chronic diseases, the COVID-19 pandemic, and healthcare demands, the clinical/POC testing market is growing, with LFIA playing a pivotal role. To enable one-step tests, LFIA uses a strip with elements such nitrocellulose membrane, conjugate pad, and sample application pad. Beyond clinical diagnostics, LFIA has applications in veterinary medicine, food safety, and environmental management and agriculture. LFIA's development and uses continue to improve with the advancement of scientific research, providing a flexible and efficient instrument for quick on-site examination. This book chapter aims to provide readers an overview of various applications of LFIAs and their future prospectives.

1. **INTRODUCTION**

Modern society is constantly at risk of various health hazards, whether they are man-made or natural, such as those caused by food chain monitoring issues, environmental contamination, and pathogens of plants and animals. Therefore, we must continually keep an eye out for both the etiological agents and our reaction to them via our elicited immunoglobulins, which are part of our adaptive immune response. There are numerous techniques for identifying pathogens, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA), cell culture and real-time polymerase chain reaction (qPCR) are examples of conventional laboratory-based analytical techniques that typically require lengthy and complex procedures to produce a result (1,2). However, enabling approaches that increase speed, sensitivity, and specificity are of utmost relevance and there is a need for fast and on-site detection. These characteristics are typically detected through point-of-care or on-site measurements. The lateral flow immunoassay (LFA), launched by Unipath in 1988, is one of the most successful systems that have been brought to market thanks to a global demand. Its popularity and ease of use make it the most widely used POC diagnostic format (3). Therefore, in recent years, scientific research has increasingly concentrated on the development and improvement of portable, cost effective, and user-friendly quick techniques of analysis for point-of-care (POC) testing. The prevalence of chronic diseases, the emergence of the Coronavirus pandemic in 2020, rising population levels, expanding pressure to cut healthcare costs, and increased demand for patient-centered treatment are all factors propelling the clinical/POC testing market forward. The global severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) pandemic is the primary factor driving the expected increased growth in 2021 and beyond (4). POC devices based on lateral flow assays (LFA) are one of the methods for qualitative and quantitative analysis that are expanding quite rapidly. LFA is carried out on a strip that has been built from several components on a plastic background. These components include the nitrocellulose membrane, adsorption pad, conjugate pad, and sample application pad. Test and control lines are further divided in the nitrocellulose membrane. Pre-immobilized reagents at various points along the strip become active upon the flow of the liquid sample. LFA combines the benefits of chromatography and biorecognition probes (5). Depending on the elements of recognition used, LFAs can be divided into 2 different types- LFIA- Lateral flow immunoassay- antibodies are exclusive recognition element & NALFA- Nucleic acid lateral flow assay- recognition element includes nucleic acids. One of the most effective analytical platforms for on-site detection of target is the Lateral Flow Immunoassay (LFIA). LFA follows the same general format as ELISA, with the exception that immobilised capture antibody or antigen is bonded onto a solid phase nitrocellulose membrane rather than a plastic well. On the test and control lines of a lateral flow strip, antibodies are used as biorecognition molecules. Through immunochemical interactions, they bind to the target analyte. The advantage in this case is that the membrane enables a one-step assay, as compared to the multiple-step ELISA. LFIA, which can be thought of as a kind of lab-in-a-hand, aim to accelerate decision making and turnaround time. As a result, the LFIA application has been rapidly expanded to other domains, such as food and feed safety, veterinary, environmental monitoring, clinical analysis, agriculture, and many others, from the detection of molecules, organisms, and (bio)markers for clinical purposes. The unique benefits of LFA strips include their rapidity and one-step analysis, low operational cost, simple instrumentation, user-friendly format, higher specificity and better sensitivity, long-term stability under various environmental conditions, and portability of the device (6). When taking into account the LFIA's place in the commercial environment, success may also be shown. In fact, the global market for lateral flow tests is expected to reach $10.36 billion in US sales by 2027, rising at a compound annual growth rate (CAGR) of 7.7 percent from 2020 to 2027. In 2019, the market was anticipated to be worth about $5.98 billion in US dollars (1). In highlighting the particular significance of LFA testing, we can draw attention to their usage in managing the global coronavirus disease (COVID-19) pandemic brought on by the novel coronavirus (SARS-CoV-2). The purpose of this review is to examine and debate the different lateral flow assay applications in the areas of food, the environment, agriculture, veterinary medicine, etc. We share our perceptions on future developments of LFIA devices is also discussed.

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**Table 1. Advantages and disadvantages of LFIA**

1. **APPLICATIONS**
2. **Clinical analysis-**

A major part of LFA applications lies in clinical analysis. It includes detection of a variety of clinical analytes in plasma, serum, urine, cells, tissues and other biological samples. The majority of clinical diagnostic techniques used today rely on central laboratory analyses that produce results in a few hours or even days. The potential advantages of LFIA application in the clinical field are self-evident given the fact that in many situations, making a quick choice can have a significant impact on the therapeutic outcome (7–9). The use of LFIA can be extremely beneficial for screening, diagnosis, prognosis, monitoring, and surveillance. The quick clinical evaluation could make a big difference in the management of the condition by alleviating workload, enhancing workflow, enhancing clinical care and patient outcomes, and possibly lowering expenses (7,9). LFIAs are inherently suitable for application in settings other than laboratories (7). LFIAs are thus utilised in hospital wards, clinics, health centres, doctors' offices, and even patients' homes in the self-testing format, in addition to those conducted by healthcare professionals in hospital laboratories (7,9,10). The direct impact on human health and the fact that the very first applications of LFIAs were for clinical usage can be attributed to the technology's tremendous success in the clinical setting. The pregnancy test is without a doubt the test that represents clinical diagnostics the best (10,11). Furthermore, it is worth noting that biological fluids are relatively few in number even when taking into account their relative complexity. For instance, venous or capillary blood, saliva, urine, nasopharyngeal swabs, and faeces are the most often utilised biological matrices (9,12,13). However, other additional LFIAs have been created and applied over time for various clinical objectives.

Rosalyn S. Yalow, a biophysicist, and Solomon A. Berson, an endocrinologist, initially proposed the idea of diagnostics based on lateral flow immunoassay (paper chromatography) in 1959. A quick test to identify insulin in human blood plasma was the first device made using paraffin paper (14). In 2015, C.S. Jorgensen and co-authors successfully demonstrated the first commercial combined LFIA test system. It enabled effective detection of *Legionella pneumophila* and *Streptococcus pneumoniae* antigens in urine. This made the versatile LFIA technology gain even more popularity (15). The first Ebolavirus outbreak, which had a mortality rate of 70.8% (16), occurred in West Africa in 2014. Using LFIA as a POC approach, IgG antibodies to the EBOV glycoprotein were 100% sensitively detected during the rapid diagnosis of EBOV(17). A recently developed multiplexed disease diagnostic strip can now identify the three distinct viruses that cause Dengue, Yellow Fever, and Ebola (18). The label utilised in this investigation was made up of AgNPs in three different sizes and colors. With a detection limit of 150 ng ml, these nanoparticles were utilised in three distinct test lines and coupled to antibodies specific for a particular disease to quickly identify the virus present in the sample. SARS-CoV-2 epidemic had a tremendous impact on public health worldwide. A serological POC kit was developed for the diagnosis of COVID-19. This makes the development of tools for the accurate diagnosis of COVID-19 important for public health. In order to create a solid-phase LFIA strip, SARS-CoV-2 nucleocapsid protein was fixed to the cassette's surface, and anti-human IgG was coupled with colloidal gold particles (19). Wu et al. performed a retrospective analysis of the dynamics of SARS-CoV-2 antibody appearance, the time-dependent sensitivity of four LFIA test systems in patients, and the importance of quick serological tests in the treatment of COVID-19 patients, whose diagnosis was made by molecular testing (RT-PCR). It came out that all tests detected antibodies (IgM and IgG) 3 weeks after the start of the disease's symptoms, and that their sensitivity and specificity were both 100%. Additionally, an earlier emergence of antibodies against SARS-CoV-2 was found in patients with COVID-19 accompanied by pneumonia (20). A new LFIA technique was developed and tested by Z. Chen and colleagues for the detection of anti-SARV-CoV-2 IgG antibodies in human serum. The recombinant nucleocapsid phosphoprotein SARS-CoV-2 and lanthanide-doped polystyrene nanoparticles, are used in the novel test system's design and are used to capture particular antibodies. The entire analysis takes ten minutes (21). Reverse transcription recombinase-aided amplification (RT-RAA) and lateral-flow dipstick (LFD) assay were combined to provide a novel point-of-care test for the detection of dengue virus (DENV) RNA (22). Globally, human noroviruses (HuNoVs) are a major contributor to nonbacterial gastroenteritis in people of all ages. An immunochromatographic assay (ICA) based on colloidal gold was created for the efficient identification of HuNoVs in clinical samples. In the ICA, shell (S) domain-specific monoclonal antibodies (MAbs) against the primary capsid protein of HuNoVs were employed (23).

1. **Foodborne pathogens and toxins**

Food quality is impacted at every stage, from transportation to processing (24,25). Food items need to be thoroughly labelled with all of their major and minor constituents. Currently, the food sector uses traditional culture-based approaches. They offer fair sensitivity and selectivity, but their main drawbacks are a cumbersome assay procedure and a lengthy analysis time (26). Since it is known that unsafe food can cause over 200 ailments, ranging from cancer to digestive tract infections, it is extremely important that risk-free food be produced and commercialised. In addition, consuming contaminated food annually causes the deaths of approx. 420,000 individuals and the illnesses of over 600 million people (4). The World Health Organization estimates that each year, 600 million people, or nearly 1 in 10 people globally, get foodborne diseases as a result of consuming contaminated food (WHO 2022). In order to prevent the inclusion of potentially hazardous chemicals such veterinary medications, heavy metals, pesticides, toxins, fertilisers, pathogens, and undeclared allergenic additives, the food safety must be assured across the entire food chain (27,28). For the identification of foodborne pathogens and toxins, quick and convenient POC approaches are required. Results are provided quickly and are "ready to read". By expanding the number of analyses, making them easily available, quick, and affordable, and enabling the monitoring of food safety along the whole production chain, from raw materials to ready-to-eat products, the use of LFIAs in food safety can aid in the management of foodborne risk. LFA or immunochromatographic strip test has been used in a variety of qualitative, semi-quantitative, and quantitative assessments (29). They are also the perfect tool to employ in the Hazard Analysis and Critical Control Points (HACCP) protocols due to their ease of use and speed (30). The public's awareness of food safety has increased over the past ten years as a result of outbreaks of foodborne illnesses from various food sources. LFIAs for the field of clinical diagnosis deal with fewer sample matrices; in contrast, LFIAs for the field of food safety must overcome a greater obstacle because food matrices can be complex and numerous (12,31). Depending on the target analyte's characteristics and the relevant matrix, it may occasionally be essential to carry out the extraction using an organic solvent. However, the tolerance for organic solvents varies between antibodies and LFIA parts, especially the nitrocellulose membrane. Determining the best solvent solution for analyte solubility and method performance may therefore be a difficult task in assay development. It is occasionally necessary to perform an additional dilution step in a suitable buffer to create a suitable medium for more effective analyte detection (32).

Botulinum neurotoxins (BoNT) are one of the deadliest neurotoxins. They are produced by spore forming obligate anaerobe, Clostridium botulinum, which occurs in the soil. There are seven different varieties of BoNts. These toxins prevent the release of acetylcholine, which causes paralysis and death. BoNT/A and B, which are known to be harmful and account for 80% of disease caused on by milk and apple juice, were targeted by highly sensitive LFA (33). Using a colloidal gold lateral flow strip, corn, feed, and wheat were tested for the simultaneous presence of the mycotoxins zearalenone and fumonisin B1. The outcomes and ELISA and LC-MS results were in good agreement (34) Recently, Salmonella enteritidis was detected using a gold nanoparticle and aptamer-based LFA, which was capable of detecting as few as 101 colony forming units (CFU) (35). For the purpose of detecting *Vibrio cholera*, freshly formed antibodies in combination with AuNPs were used in LFA (36). For the purpose of quantifying Salmonella, a nucleic acid lateral flow test was developed. Gold nanoparticles were coupled with a DNA probe that was highly specific to salmonella DNA and 16s ribosomal RNA. Deposition of silver improved the signal (37). In respiratory samples obtained from people with extremely severe asthma using LFA, the detection limit of 106 cfu/mL for Staphylococcus aureus was attained. The test indicated high pathogen specificity (38). Using fluorescent nanosilica, LFA was utilised to detect clenbuterol in urine of animals which causes disorders of heart and nervous system. The visual detection limit for qualitative analysis was determined to be 0.1 ng/mL, and the limit of detection for quantitative analysis was as low as 0.037 ng/mL (39). An allergic reaction may result from the ultra-minor presence of crustacean protein in processed meals. To identify the presence of crustacean protein in processed foods, a strip with a very low optical detection limit was developed (40). When excessive amounts of the veterinary medication sulfamethazine (SMZ), which is frequently used in animal husbandry, are found in food, they are detrimental to human health. Time-resolved fluorescent nanobeads (TRFN) were used as a label for the development of an immunochromatographic assay (ICA) that is quick, reliable, and accurate for the detection of SMZ in samples of eggs, honey, and pork (41). Two immunochromatographic test strips were effectively developed using two Au nanomaterials—colloidal Au Sphere and Nano Rods—for the visual detection of Zearalenone (ZEN) in cereals (42). For the quantitative detection of Salmonella spp., a quick and reliable lateral flow fluorescent immunoassay based on strand exchange amplification (SEA-LFIA) was created. The SEA-LFIA assay had a sensitivity of 6 100 CFU mL-1 for pure Salmonella or 3 104 CFU 25 g-1 for intentionally spiked raw chicken flesh (43).

1. **Veterinary**

Veterinary medicine primarily treats cattle and companion animals, or animals that are valued as assets, such as dogs, cats, and other pets (cows, sheep, poultry, pigs, etc.). Due to pet owners' desire to keep their pets healthy and farmers' growing awareness of the advantages of near-animal testing, the usage of diagnostic quick tests in the veterinary industry has expanded over the past few decades. The growing consumer concern about antibiotics, transmissible diseases in milk, eggs, and meat, as well as the general public concern over the spread of diseases through populations of animals, are further factors driving the acceptance of diagnostic quick tests in veterinary medicine (44). The provision of veterinary services is crucial for ensuring animal health and, in a broader sense, for the prevention and control of animal diseases, including those that can be transmitted to humans (zoonoses), for ensuring the sanitary safety of international trade in terrestrial and aquatic animals as well as animal products, and for improving animal welfare on a global scale (32). The exponential development in trade and tourism now makes it possible for animal diseases to spread even further. To reduce their detrimental effects, the disease identification and outbreak report must be made as quickly as feasible. Of course, infectious diseases pose the greatest threat due to their ease of transmission and severe effects (large-scale culling of livestock in some cases). Rapid screening tests may facilitate and speed up the diagnosis of infectious diseases in this situation, enabling fast and targeted intervention to stop the spread of the disease. Furthermore, it is simple to comprehend the critical importance of the early detection of these diseases at their source in animals given that 60% of the infections that impact humans are of animal origin (32,45). The key to preventing and controlling animal diseases is effective surveillance, early detection, transparency, and rapid response systems in the event of disease outbreaks. Immediate response times result in better management and intervention techniques. Currently, veterinarians examine domestic pets and commercial cattle for a variety of medical issues using quick tests. These tests are useful in a variety of veterinary settings, such as private clinics, academic veterinary medical centres, remote communities, and research applications in academia, government, and business (46) Due to the enormous diversity of animal species and their unique characteristics, LFIAs for veterinary use have to deal with a wide range of matrices, including blood, urine, buccal and nasal secretions, mammary secretions, milk, faeces, respiratory exhalations, etc (32).

For the quantitative detection of the avian leukosis virus (ALV), a quick fluorescence microsphere immunochromatographic test strip (FM-ICTS) assay was developed (47). Evaluation and development of a protein-G-based lateral flow assay (LFA) for quick serodiagnosis of brucellosis in several domesticated animal species. Results demonstrated that LFA has a sensitivity and specificity of 89 and 99 percent, respectively (48). Based on anti- *Infectious Bursal Disease Virus* (IBDV) IgY as the bio receptor, the lateral flow immunoassay was effectively developed. Isa Brown's egg yolk was used to isolate anti- IBDV IgY (49). A quick flow-through immunoassay for the qualitative detection of trypanosomosis in equine serum samples, using protein a labelled gold nanoparticles (GNPs) was created. The assay can be finished in under 5 minutes. The flow-through assay's outcomes were equivalent to those of ELISA and dot blot assays (50). Sport horses are more susceptible to infectious infections when they are transported. In an investigation, rectal temperature was compared to serum amyloid A (SAA) as a marker of early inflammation in sporthorses after air travel. A stall-side lateral flow immunoassay was used to evaluate SAA (51). Another experiment was conducted to create and validate a tool for calculating circulating plasma concentrations of progesterone (P4) in bovine plasma, that combines a disposable fluorescence-based lateral flow immunoassay (LFIA) paired with a portable imaging device (52). The main cause of human visceral leishmaniasis is canine visceral leishmaniasis (CVL). Early and precise diagnosis of affected dogs is difficult but necessary in order to control the spread of this disease. As a visual in situ technique for the diagnosis of CVL, a lateral flow immunoassay (LFIA) based on functionalized coloured particles and a particular recombinant antigen was designed, optimised, and standardised (53). The pig industries suffer considerable financial losses as a result of porcine epidemic diarrhoea (PED), which is brought on by the porcine epidemic diarrhoea virus (PEDV). PED causes acute diarrhoea, vomiting, dehydration, and high mortality in neonatal piglets. For the quick detection of PEDV, an immunochromatographic assay (ICA) based on a EuNPs-mAb fluorescent probe was created. The ICA's linear detection range was 0.03125-8 g/mL, and its limit of detection (LOD) was 0.218 g/mL (54).

1. **Environment**

Environmental contamination has emerged as a critical global issue. Many toxins and pollutants enter the environment either as a result of anthropogenic activity like industry, agriculture, transportation, daily activities, etc., or as a result of naturally occurring events (55). Pollutants and contaminants can travel from one medium to another through the air, the soil, or the water (for example, soil to water). They may have a direct or indirect impact on a nation's socioeconomic development and on people's health (56). Emerging contaminants (ECs) have gained importance over the past few years, and as a result, more and more substance classes have to be monitored in order to protect the environment. ECs cover a broad range of substances, including medications, personal care items, endocrine disrupting substances, sweeteners, nanoparticles, etc (57,58). Antimicrobials are a growing source of concern among ECs because of the possibility that microbials develop resistance against drugs and accumulate in animals (32). In order to anticipate clinically relevant infection outbreaks, it is necessary to monitor the environment for antibiotic resistance species (59). Consequently, identifying and keeping an eye on air, soil, and water pollutants is of utmost importance. The monitoring of pollutants enables the identification of the spatial distribution of contaminants to identify which locations are at danger as well as the examination of temporal trends at various sites to ascertain if the situation is becoming better or worse (1). Due to the size of the environmental media, controlling the amount of contaminants in the environment is expensive, labour-intensive, and frequently time-consuming. Environmental analyses involve extensive knowledge of advanced analytical chemistry, as well as sophisticated and costly apparatus (55). Pathogens are typically identified using polymerase chain reaction (PCR)-based detection, while pollutants are detected in the lab using chromatographic and spectroscopic approaches (60). In order to execute a cost-effective monitoring, alternative ways that can quickly and easily deliver on-site, high-throughput, simple, and real-time testing are highly desired (61). When evaluating inorganic and organic pollutants as well as biological contaminants, LFIAs can be employed as monitoring tools for environmental quality (Parolo et al., 2020). Although this type of sensor is not typically used to monitor air quality, it is mostly utilised to monitor water and soil-borne contaminants (Marquez et al., 2019; Parolo et al., 2020).

A colloidal gold-strip assay was created to simultaneously measure carbofuran (CBF) and its metabolite 3-hydroxy-carbofuran (3-OH-CBF) based on a broad-specific monoclonal antibody (mAb). The colloidal gold-strips perform with a cut-off limit of detection (LOD) of 7–10 ng/mL for carbofuran and 3-OH-CBF and have a runtime of less than 5 min without complex sample pretreatment (62). Poisoning with paraquat (PQ) poses a major risk to human health since it can cause inflammation, neurotoxicity, and lung toxicity. PQ concentrations in water samples were determined using two monoclonal antibodies against PQ and an immunochromatographic assay (ICA) (63). Human adenovirus (HAdV), a viral water quality indicator, may now be detected quickly using new tests. To distinguish between HAdV A, B, C, and F, species-specific assays were created and merged into a multiplex test (64). Using phenylboronic acid as a chelating agent and oligocytosine chain as a receptor for the generated complexes, a lead (II) lateral flow test strip was developed and approved. The test time was 5 minutes, and the method is characterised by high sensitivity (0.05 ng mL1) and the lack of cross-reactions with other metal ions (65). For the detection of E. coli O157:H7 in beef and river water, a single antibody-based fluorescent lateral flow immunoassay (FLFIA) based on non-radiative energy transfer between graphene oxide and quantum dots was developed. The assay cost was 60% less with the single antibody method compared with the traditional LF. Additionally, cellphones or portable LF readers could read the results (66) A test strip was created to using lateral flow assay to quickly find bisphenol A in snow. Bisphenol A could be detected at concentrations as low as 0.1 pg/mL instrumentally and 20 ng/mL visually (67). The poisonous microalgae metabolites known as microcystins have negative impacts on both human and environmental health. For the purpose of detecting the most prevalent microcystin variant, microcystin-LR (MC-LR), three immunochromatographic test formats were created and compared. The indirect labelling formats yielded the best sensitivity and stability (68). Karenia mikimotoi, dinoflagellate is a noxious and harmful algal bloom (HAB)-forming microalga. Hyperbranched rolling circle amplification (HRCA) paired with lateral flow dipstick (LFD), often known as HRCA-LFD, is an isothermal amplification method combined with a quick analytical method for nucleic acid-based amplified products was developed to identify K. mikimotoi (69).

1. **Agriculture**

In order to meet the demands of rising population during the last 300 years, agricultural ecosystems have expanded to cover over 40% of the planet's surface (70). One of the greatest limits on food supplies around the world is the deterioration of plant products, which is estimated to cause a loss of between 10 and 30 percent overall (71). The proliferation of phytopathogens has surged in recent decades, with the most detrimental effects (72). It is linked to both climatic and environmental changes as well as the phytopathogens' own adaptive modifications (73,74). Fungi, bacteria, and viruses make up the top ten list of commercially and scientifically significant plant pathogens (75–78). Monitoring the health of plants requires early detection of plant diseases. Different sensing techniques, ranging from the most basic detection of symptoms appearing on leaves to nucleic acid detection methods, have been used throughout the years to build sensitive and selective detection systems. Several earlier studies focused on identifying plant pathogens and diagnosing plant diseases utilising nucleic acid-based techniques, primarily polymerase chain reaction (PCR) and DNA hybridization detection to identify the genetic makeup of pathogens (79–82). Some techniques include detection of pathogens using antibodies like ELISA (83–85). However, these described techniques must be carried out in a lab by qualified personnel utilising costly apparatus. Innovative and portable biosensors have become widely employed as diagnostic instruments in plant pathogen detection in recent years to overcome these constraints. Isothermal DNA amplification techniques, in particular, are frequently utilised for plant pathogen detection and utilised in several LFA versions (86,87). Lateral flow immunoassay is primarily used to give such quality as quick on-site analysis (LFIA) (88). It has been demonstrated that plant pathogens can be detected using LFIA (89). However, there is still a need for pathogen identification in the field, utilising a test that can quickly and accurately determine whether a specific pathogen is present or absent in symptomatic tissue.

For *Xanthomonas campestris* pv. *musacearum* detection, a polyclonal LFIA has been created. All strains of *X. campestris* pv. *musacearum* can be detected using this assay, but also showed cross-reactivity to *X. axonopodis* pv. *vasculorum*. This test's sensitivity level was set at 105 CFU/ml (90). Comparable specifications were found in a similar test to identify Xanthomonas arboricola pv. pruni, which had great specificity (only displaying cross-reactivity against X. arboricola pv. corylina) and a sensitivity of 104 CFU/ml (91). The EPPO also advises using LFIAs to find plant pathogenic viruses such the watermelon silver mottle virus, impatiens necrotic spot virus, and tomato spotted wilt virus. To prevent false-positive results, EPPO further suggests that positive LFIA tests require confirmatory validation using ELISA or PCR-based techniques (92). A recombinant coat protein of the banana bract mosaic virus (BBrMV) was generated in E. coli in order to make polyclonal antibody against it. The expressed BBrMVcoat protein had a LOD of 10 ng, and the crude extract had a detection limit of 1:20 dilution with an action time of 5 to 10 minutes (93). For the identification of the Citrus tristeza virus (CTV), a quick and highly targeted diagnostic method was created. The procedure combines a lateral flow immunochromatographic assay (LFCIA) with reverse transcription-recombinase polymerase amplification (RT-RPA). The CTV-p25 gene is amplified successfully by the improved RT-RPA-LFICA procedure, and double-labeled amplicons are found using a sandwich immunoassay. It exhibits great sensitivity and specificity without cross-reactivity and can detect as little as 141 fg of RNA or 0.23 ng/l of CTV RNA (94). Another LFIA was developed for the quick detection of the fire blight-causing bacterium *Erwinia amylovora*, which affects plants of the Rosaceae family. The LFIA showed good specificity and efficacy, quickly identifying E. amylovora in plant samples. Multiple samples taken from the same plant considerably improved the accuracy of detection when different plant organs were evaluated. Comparison of the LFIA's performance with PCR-based kits proved its efficacy (95). Ivanov et al. developed a test to identify the plant pathogen PSTVd, or potato spindle tuber viroid, which harms crops. The technique combined lateral flow assay (LFA) for amplicon identification with reverse transcription and recombinase polymerase amplification (RT-RPA) of PSTVd RNA. When analysing healthy and infected potato samples, RT-RPA-LFA exhibited complete concordance with RT-qPCR and a commercial kit, demonstrating its potential for PSTVd detection. It detected 106 copies of PSTVd RNA in 30 minutes (96). For the precise diagnosis of Dickeya solani-caused potato blackleg disease, lateral flow assays (LFA) and isothermal DNA amplification were established. Using a primer pair matched to the SOL-C region of the D. solani genome and flanked by fluorescein and biotin, recombinase polymerase amplification (RPA) was employed. The assay offered sensitivity comparable to PCR at a constant temperature and short time, with a detection limit of 14,000 colony-forming units per gramme of potato tuber (97).

1. **Conclusion**

In clinical analysis, food safety, agriculture, veterinary and environmental analysis, point of care (POC) testing has emerged as the most well-known method of diagnosis. POC provides prompt outcomes in lesser time as compared to centralised labs. Therefore, on-site quick diagnostic techniques that are robust, efficient, sensitive, and cost-effective are urgently needed to speed up the detection of pathogens. Among various immunoassay-based analytical platforms, one of the most effective analytical platforms for point-of-care or decentralised testing strategies requiring little to no supporting infrastructure is the lateral flow immunoassay technique (LFIA), also known as immunochromatographic strip test (ICST), or rapid diagnostic test (RDT). The LFIA is a paper-based (bio)analytical method for on-site detection of target compounds, where the sample is added on a standalone device and the outcome is acquired in a short period of time. LFIAs met all the requirements for an ideal POCT that must be "ASSURED" (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Delivered) (98). LFIAs can be carried out by untrained personnel and do not involve pipetting or washing procedures. Additionally, there is no need for a cold chain. Gold or silver nanoparticles are mostly used to label the secondary antibodies. Within 10 to 30 minutes, LFIAs can identify pathogen-specific antigens and/or antibodies. Multiple targets can now be detected in a single test using multiplex LFIAs that have recently been developed (99).

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