LATERAL FLOW IMMUNOASSAYS: THE FUTURE OF ON-SITE DETECTION AND DIAGNOSTICS

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 provides readers an overview of various applications of LFIAs and its future prospectives.

Keywords: LFIAs, POC, Diagnostics

Authors

Kritika Shukla
Plant Molecular Virology Lab
Department of Botany
School of Chemical and Life Sciences
Jamia Hamdard, New Delhi, India.

Nikita
Plant Molecular Virology Lab
Department of Botany
School of Chemical and Life Sciences
Jamia Hamdard, New Delhi, India.

Md Salik Noorani
Plant Molecular Virology Lab
Department of Botany
School of Chemical and Life Sciences
Jamia Hamdard, New Delhi, India.
saliknoorani@jamiahamdard.ac.in

I. 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 been concentrating on the development and improvement of on-site, cost effective, and easy-to-use techniques 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 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 (LFAs) are one of the methods for qualitative and quantitative analysis that are increasing rapidly. LFA is carried out on a strip that has been built on a plastic background.

Its various components include the nitrocellulose membrane, adsorption pad, conjugate pad, and sample application pad. Test and control lines are further divided in the nitrocellulose membrane. Upon the flow of the liquid sample, pre-immobilized reagents become active at various points on the test strip. LFA combines the benefits of chromatography and biorecognition probes (5). Depending on the recognition element used, LFAs can be divided into 2 different types- LFIA- Lateral flow immunoassay, where the recognition elements are antibodies & NALFA- Nucleic acid lateral flow assay, where 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 instead of plastic well, immobilised capture antibody or antigen is bonded onto a 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 applications are expanding rapidly to various fields such as, food technology, veterinary, environmental sciences, clinical analysis, agriculture etc. The unique benefits of LFA strips

include their speed 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 $13.38 billion in US sales by 2030, rising at a compound annual growth rate (CAGR) of 4.7 percent from 2022 to 2030. In 2022, the market was anticipated to be worth about $8.49 billion in US dollars (7). In highlighting the particular significance of LFA testing, we can draw attention to their usage in managing the global coronavirus disease (COVID-19). The purpose of this review is to examine and debate the different applications of lateral flow assay in the areas of food, environment, agriculture, veterinary, medicine, etc. Our perceptions on future developments of LFIA devices is also discussed.

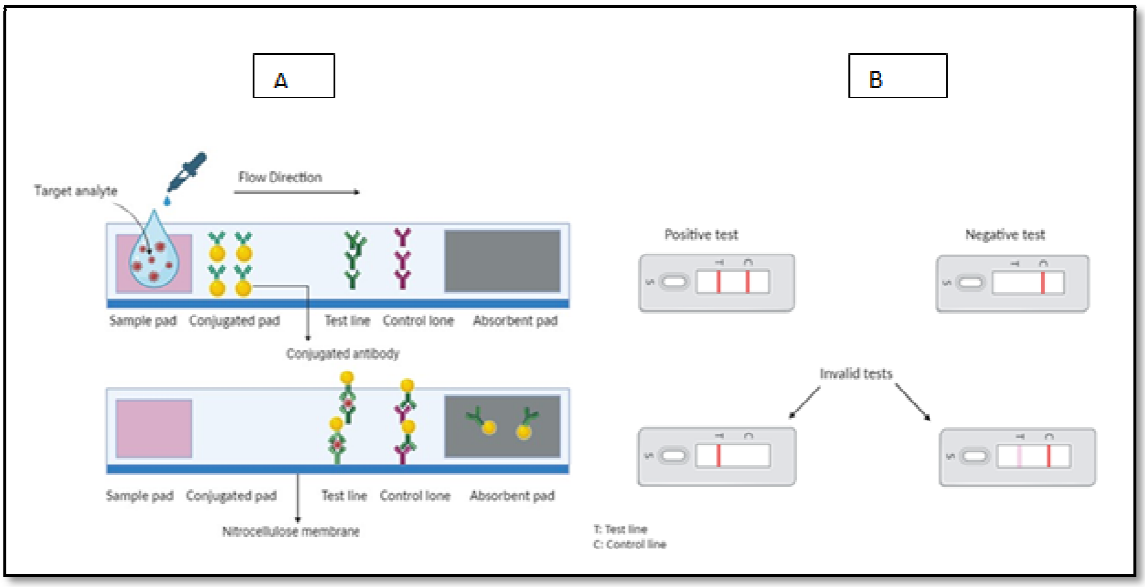

Figure 1: The operation principle of lateral flow immunoassay (LFIA) sandwich-based method.

(A) Schematic illustration of a typical LFIA test strip. The sample containing the target analyte is applied unto the sample pad and it moves along the lateral flow strip towards conjugated pad containing conjugated antibodies (usually AuNP-conjugated) via capillary action. The antigens in the sample interact with the conjugate, and both migrate to the next section of strip, where the antigen/antibodies are immobilized. Here, the analyte and conjugate are captured. Excess reagent passes through and accumulates on the absorbant pad.

(B) The results are interpreted on the nitrocellulose membrane by the presence or absence of the test and control lines. A positive test is indicated by two bands, whereas a negative test is indicated by a single control band. An invalid result is indicated by no band or just one band at the test line.

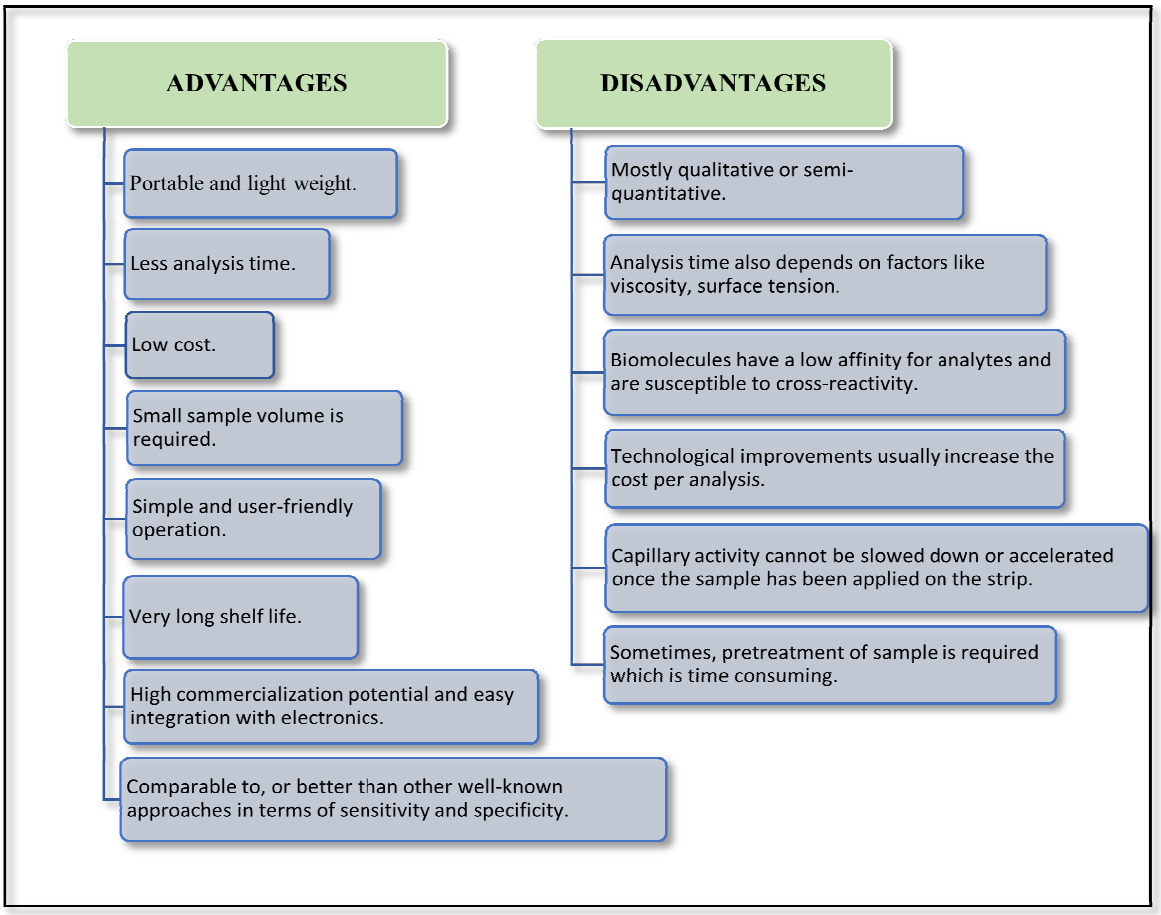

Figure 2: Advantages and disadvantages of Lateral flow Immunoassay (LFIA).

II. APPLICATIONS

1. **Clinical Analysis:** Clinical analysis constitutes the major part of applications of LFAs. Variety of clinical analytes can be detected 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 (8–10). 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 reducing workload, better workflow, enhancing clinical care and patient outcomes, and possibly lowering expenses (8,10). LFIAs are inherently suitable for application in settings other than laboratories (8). LFIAs are thus utilised in hospitals, clinics, health centres, and even in patients' homes in the self-testing format, in addition to those conducted by healthcare professionals in hospital laboratories (8,10,11). 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 (11,12). Furthermore, it is worth noting that biological fluids are relatively few in number even when taking into account their relative complexity. For instance, saliva, blood, nasopharyngeal swabs, urine, and

faeces are the most often utilised biological matrices (10,13,14). 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 (15). 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 (16). The first Ebolavirus outbreak, which had a mortality rate of 70.8% (17), 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(18). A recently developed multiplexed disease diagnostic strip can now identify the three distinct viruses that cause Yellow Fever, Dengue, and Ebola (19). 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. The SARS-CoV-2 epidemic had a major impact on global public health. 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 (20). Wu et al. performed an 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 (21). 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 entire analysis takes ten minutes (22). 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 (23). Globally, human noroviruses (HuNoVs) are a major contributor to nonbacterial gastroenteritis in people of all ages. An immunochromatographic assay (ICA) 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 (24).

2. **Foodborne Pathogens and Toxins:** Food quality is impacted at every stage, from transportation to processing (25,26). 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 (27). It is extremely important

that risk-free food be produced and commercialised as contaminated food can cause over 200 ailments ranging from cancer to digestive tract infections. In addition, consuming contaminated food annually causes the deaths of approx. 420,000 individuals and the illnesses of over 600 million people (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". 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 (13,31). 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 produced by spore forming obligate anaerobe, *Clostridium botulinum*, which occurs in the soil. These toxins prevent the release of acetylcholine, which causes paralysis and death. Of 7 different variants of BoNTs, 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). 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) (34). For the purpose of detecting *Vibrio cholera*, freshly formed antibodies in combination with AuNPs were used in LFA (35). 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 (36). 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 (37). 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 (38). 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 (39). 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 (40). 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 ×

10^0 CFU mL^{-1} for pure *Salmonella* or 3×10^4 CFU 25 g^{-1} for intentionally spiked raw chicken flesh (41).

3. **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.). The use of POC diagnostics in the veterinary sector has increased over the past few decades as a result of pet owners' desire to maintain the health of their animals and farmers' increasing understanding of the benefits of near-animal testing. Another reason influencing the acceptability of diagnostic tests in veterinary medicine is the increased consumer concern about antibiotics, transmissible diseases in milk, eggs, and meat, as well as the general public's concern about the spread of diseases via populations of animals (42). 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. 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 (32,43). 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.

   For the quantitative detection of the *avian leukosis virus* (ALV), a quick fluorescence microsphere immunochromatographic test strip (FM-ICTS) assay was developed (44). 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 (45). 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 (46). 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 (47). 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 (48). 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 (49). 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 (50). 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 (51).

4. **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 agriculture, transportation, industries, daily activities, etc., or as a result of naturally occurring events (52). 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 (53). 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 (54,55). 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 (56). Consequently, identifying and keeping an eye on air, soil, and water pollutants is of utmost importance.

   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 (52). Pathogens are typically identified using polymerase chain reaction (PCR)-based detection, while pollutants are detected in the lab using chromatographic and spectroscopic approaches (57). 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 (58). 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 (59). Poisoning with paraquat (PQ) 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) (60). 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 (61). A lead (II) lateral flow test strip was created and validated using phenylboronic acid as a chelating agent and oligocytosine chain as a receptor for the complexes produced. 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 (62). A single antibody-based fluorescent lateral flow immunoassay (FLFIA)

based on non-radiative energy transfer between graphene oxide and quantum dots was created for the detection of E. coli O157:H7 in beef and river water. 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 (63) 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 (64). 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 (65).

5. **Agriculture:** Over the last 300 years, agricultural ecosystems have increased to cover more than 40% of the surface of the earth in order to meet the demands of a growing population (66). The degradation of plant products, which is estimated to result in a loss of between 10 and 30 percent overall, is one of the biggest constraints on food supplies around the world (67). The proliferation of phytopathogens has surged in recent decades, with the most detrimental effects (68). It is linked to both climatic and environmental changes as well as the phytopathogens' own adaptive modifications (69,70). Fungi, bacteria, and viruses make up the top ten list of commercially and scientifically significant plant pathogens (71–74). Monitoring the health of plants requires early detection of plant diseases. Over time, several sensing methods have been employed to develop sensitive and specialized detection systems, from the simplest detection of symptoms to nucleic acid detection approaches. Several earlier studies focused on identifying plant pathogens and diagnosing plant diseases utilising nucleic acid-based techniques, primarily PCR and DNA hybridization detection to identify the genetic makeup of pathogens (75–78). Some techniques include detection of pathogens using antibodies like ELISA (79–81).

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 (82,83). Lateral flow immunoassay is primarily used to give such quality as quick on-site analysis (LFIA) (84). It has been demonstrated that plant pathogens can be detected using LFIA (85). 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.

A polyclonal LFIA has been developed for the detection of Xanthomonas campestris pv. musacearum. This assay can detect all strains of X. campestris pv. musacearum and also demonstrated cross-reactivity to X. axonopodis pv. vasculorum. The sensitivity level for this test was set at 105 CFU/ml (86). 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 (87). The EPPO also advises using LFIAs to find plant pathogenic viruses such the watermelon silver mottle 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 (88). 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 (89). For the identification of the Citrus tristeza virus (CTV), a quick and highly targeted diagnostic method was created. The procedure combines an LFCIA with 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 (90). Another LFIA was developed for the quick detection of the bacterium *Erwinia amylovora*, that causes fire blight and 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 (91). Ivanov et al. developed a test to identify the plant pathogen PSTVd, or potato spindle tuber viroid, which harms crops.

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 (92). For the precise diagnosis of *Dickeya solani*-caused potato blackleg disease, lateral flow assays 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, with a detection limit of 14,000 CFU/g of potato tuber (93).

6. **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, it is critically necessary to develop on-site fast diagnostic tools that are reliable, effective, sensitive, and economical. The lateral flow immunoassay technique (LFIA), also known as the immunochromatographic strip test (ICST), is one of the most efficient analytical platforms for point-of-care necessitating little to no supporting infrastructure. The LFIA is a paper-based (bio)analytical approach for the rapid acquisition of results from the on-site detection of target substances. The sample is added to a standalone device. The ideal POCT must be "ASSURED" (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Delivered), and LFIAs satisfied all of these criteria (94). LFIAs do not require pipetting or washing procedures and can be performed by inexperienced persons. Additionally, a cold chain is not required. The secondary antibodies are typically labelled using gold or silver nanoparticles. LFIAs can recognise pathogen-specific antigens and/or antibodies in 10 to 30 minutes. With the recent development of multiplex LFIAs, multiple targets can now be detected in a single test (95).

REFERENCES

[1] Campbell VR, Carson MS, Lao A, Maran K, Yang EJ, Kamei DT. Point-of-Need Diagnostics for Foodborne Pathogen Screening. SLAS TECHNOLOGY: Translating Life Sciences Innovation [Internet]. 2020 Oct 5;26(1):55–79. Available from: https://doi.org/10.1177/2472630320962003
[2] Charlermroj R, Phuengwas S, Makornwattana M, Sooksimuang T, Sahasithiwat S, Panchan W, et al. Development of a microarray lateral flow strip test using a luminescent organic compound for multiplex detection of five mycotoxins. Talanta [Internet]. 2021;233:122540. Available from: https://www.sciencedirect.com/science/article/pii/S0039914021004616
[3] Davies RJ, Eapen SS, Carlisle SJ. Lateral-Flow Immunochromatographic Assays. In: Handbook of Biosensors and Biochips [Internet]. 2007. Available from: https://doi.org/10.1002/9780470061565.hbb110
[4] Boehringer HR, O'Farrell BJ. Lateral Flow Assays in Infectious Disease Diagnosis. Clin Chem [Internet]. 2022 Jan 1;68(1):52–8. Available from: https://doi.org/10.1093/clinchem/hvab194
[5] Sajid M, Kawde AN, Daud M. Designs, formats and applications of lateral flow assay: A literature review. Journal of Saudi Chemical Society [Internet]. 2015;19(6):689–705. Available from: https://www.sciencedirect.com/science/article/pii/S131961031400129X
[1] Li Z, Wang Y, Wang J, Tang Z, Pounds JG, Lin Y. Rapid and Sensitive Detection of Protein Biomarker Using a Portable Fluorescence Biosensor Based on Quantum Dots and a Lateral Flow Test Strip. Anal Chem [Internet]. 2010 Aug 15;82(16):7008–14. Available from: https://doi.org/10.1021/ac101405a
[2] Global Lateral Flow Assay Market Size By Type (Lateral Flow Readers, Kits And Reagents), By Technique (Competitive Assay, Sandwich Assay), By Application (Veterinary Diagnostics, Clinical/Point-Of-Care Testing), By End-User (Home Care, Diagnostic Laboratories, Hospitals And Clinics), By Geographic Scope And Forecast. 2023 Mar.
[3] Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-Care Testing for Infectious Diseases: Diversity, Complexity, and Barriers in Low- And Middle-Income Countries. PLoS Med [Internet]. 2012 Sep 4;9(9):e1001306-. Available from: https://doi.org/10.1371/journal.pmed.1001306
[4] Parolo C, Merkoçi A. Paper-based nanobiosensors for diagnostics. Chem Soc Rev [Internet]. 2013;42(2):450–7. Available from: http://dx.doi.org/10.1039/C2CS35255A
[5] Soh JH, Chan HM, Ying JY. Strategies for developing sensitive and specific nanoparticle-based lateral flow assays as point-of-care diagnostic device. Nano Today [Internet]. 2020;30:100831. Available from: https://www.sciencedirect.com/science/article/pii/S1748013219305420
[6] Urusov AE, Zherdev A V, Dzantiev BB. Towards Lateral Flow Quantitative Assays: Detection Approaches. Biosensors (Basel) [Internet]. 2019;9(3). Available from: https://www.mdpi.com/2079-6374/9/3/89
[7] Weihs F, Anderson A, Trowell S, Caron K. Resonance Energy Transfer-Based Biosensors for Point-of-Need Diagnosis—Progress and Perspectives. Sensors [Internet]. 2021;21(2). Available from: https://www.mdpi.com/1424-8220/21/2/660
[8] Parolo C, Sena-Torralba A, Bergua JF, Calucho E, Fuentes-Chust C, Hu L, et al. Tutorial: design and fabrication of nanoparticle-based lateral-flow immunoassays. Nat Protoc [Internet]. 2020;15(12):3788–816. Available from: https://doi.org/10.1038/s41596-020-0357-x
[9] Yamada K, Shibata H, Suzuki K, Citterio D. Toward practical application of paper-based microfluidics for medical diagnostics: state-of-the-art and challenges. Lab Chip [Internet]. 2017;17(7):1206–49. Available from: http://dx.doi.org/10.1039/C6LC01577H
[10] Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest. 1960;39(7):1157–75.
[11] Jørgensen CS, Uldum SA, Sørensen JF, Skovsted IC, Otte S, Elverdal PL. Evaluation of a new lateral flow test for detection of Streptococcus pneumoniae and Legionella pneumophila urinary antigen. J Microbiol Methods [Internet]. 2015;116:33–6. Available from: https://www.sciencedirect.com/science/article/pii/S0167701215300051
[12] Ebola Virus Disease in West Africa — The First 9 Months of the Epidemic and Forward Projections. New England Journal of Medicine [Internet]. 2014;371(16):1481–95. Available from: https://doi.org/10.1056/NEJMoa1411100
[13] Brangel P, Sobarzo A, Parolo C, Miller BS, Howes PD, Gelkop S, et al. A Serological Point-of-Care Test for the Detection of IgG Antibodies against Ebola Virus in Human Survivors. ACS Nano [Internet]. 2018 Jan 23;12(1):63–73. Available from: https://doi.org/10.1021/acsnano.7b07021
[14] Yen CW, de Puig H, Tam JO, Gómez-Márquez J, Bosch I, Hamad-Schifferli K, et al. Multicolored silver nanoparticles for multiplexed disease diagnostics: distinguishing dengue, yellow fever, and Ebola viruses. Lab Chip [Internet]. 2015;15(7):1638–41. Available from: http://dx.doi.org/10.1039/C5LC00055F

[15] Wen T, Huang C, Shi FJ, Zeng XY, Lu T, Ding SN, et al. Development of a lateral flow immunoassay strip for rapid detection of IgG antibody against SARS-CoV-2 virus. Analyst [Internet]. 2020;145(15):5345–52. Available from: http://dx.doi.org/10.1039/D0AN00629G
[16] Wu JL, Tseng WP, Lin CH, Lee TF, Chung MY, Huang CH, et al. Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. Journal of Infection [Internet]. 2020;81(3):435–42. Available from: https://www.sciencedirect.com/science/article/pii/S0163445320304047
[17] Chen Y, Chan KH, Hong C, Kang Y, Ge S, Chen H, et al. A highly specific rapid antigen detection assay for on-site diagnosis of MERS. Journal of Infection [Internet]. 2016 Jul 1;73(1):82–4. Available from: https://doi.org/10.1016/j.jinf.2016.04.014
[18] Xiong Y, Luo Y, Li H, Wu W, Ruan X, Mu X. Rapid visual detection of dengue virus by combining reverse transcription recombinase-aided amplification with lateral-flow dipstick assay. International Journal of Infectious Diseases [Internet]. 2020;95:406–12. Available from: https://www.sciencedirect.com/science/article/pii/S1201971220302083
[19] Xu M, Lu F, Lyu C, Wu Q, Zhang J, Tian P, et al. Broad-range and effective detection of human noroviruses by colloidal gold immunochromatographic assay based on the shell domain of the major capsid protein. BMC Microbiol [Internet]. 2021;21(1):22. Available from: https://doi.org/10.1186/s12866-020-02084-z
[20] Utrera M, Morcuende D, Estévez M. Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. LWT - Food Science and Technology [Internet]. 2014;56(1):62–8. Available from: https://www.sciencedirect.com/science/article/pii/S0023643813003952
[21] Galindo A, Calín-Sánchez Á, Collado-González J, Ondoño S, Hernández F, Torrecillas A, et al. Phytochemical and quality attributes of pomegranate fruits for juice consumption as affected by ripening stage and deficit irrigation. J Sci Food Agric [Internet]. 2014 Aug 1;94(11):2259–65. Available from: https://doi.org/10.1002/jsfa.6551
[22] Gracias KS, McKillip JL. A review of conventional detection and enumeration methods for pathogenic bacteria in food. Can J Microbiol [Internet]. 2004 Nov 1;50(11):883–90. Available from: https://doi.org/10.1139/w04-080
[23] Organization WH. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization; 2015.
[24] Nuntawong P, Putalun W, Tanaka H, Morimoto S, Sakamoto S. Lateral flow immunoassay for small-molecules detection in phytoproducts: a review. J Nat Med [Internet]. 2022;76(3):521–45. Available from: https://doi.org/10.1007/s11418-022-01605-6
[25] Di Nardo F, Anfossi L. Chapter Eight - Commercial biosensors for detection of food additives, contaminants, and pathogens. In: Sezgintürk MK, editor. Commercial Biosensors and Their Applications [Internet]. Elsevier; 2020. p. 183–215. Available from: https://www.sciencedirect.com/science/article/pii/B9780128185926000086
[26] Anfossi L. Immunoassays| food applications. 2019;
[27] Di Nardo F, Chiarello M, Cavalera S, Baggiani C, Anfossi L. Ten Years of Lateral Flow Immunoassay Technique Applications: Trends, Challenges and Future Perspectives. Sensors [Internet]. 2021;21(15). Available from: https://www.mdpi.com/1424-8220/21/15/5185
[28] Ching KH, Lin A, McGarvey JA, Stanker LH, Hnasko R. Rapid and selective detection of botulinum neurotoxin serotype-A and -B with a single immunochromatographic test strip. J Immunol Methods [Internet]. 2012;380(1):23–9. Available from: https://www.sciencedirect.com/science/article/pii/S0022175912000907
[29] Fang Z, Wu W, Lu X, Zeng L. Lateral flow biosensor for DNA extraction-free detection of salmonella based on aptamer mediated strand displacement amplification. Biosens Bioelectron [Internet]. 2014;56:192–7. Available from: https://www.sciencedirect.com/science/article/pii/S0956566314000189
[30] Chen W, Zhang J, Lu G, Yuan Z, Wu Q, Li J, et al. Development of an immunochromatographic lateral flow device for rapid diagnosis of Vibrio cholerae O1 serotype Ogawa. Clin Biochem [Internet]. 2014;47(6):448–54.Available from: https://www.sciencedirect.com/science/article/pii/S0009912013006073
[31] Liu CC, Yeung CY, Chen PH, Yeh MK, Hou SY. Salmonella detection using 16S ribosomal DNA/RNA probe-gold nanoparticles and lateral flow immunoassay. Food Chem [Internet]. 2013;141(3):2526–32. Available from: https://www.sciencedirect.com/science/article/pii/S0308814613006821

[32] Wiriyachaiporn S, Howarth PH, Bruce KD, Dailey LA. Evaluation of a rapid lateral flow immunoassay for Staphylococcus aureus detection in respiratory samples. Diagn Microbiol Infect Dis [Internet]. 2013;75(1):28–36. Available from: https://www.sciencedirect.com/science/article/pii/S0732889312003896
[33] Koizumi D, Shirota K, Akita R, Oda H, Akiyama H. Development and validation of a lateral flow assay for the detection of crustacean protein in processed foods. Food Chem [Internet]. 2014;150:348–52. Available from: https://www.sciencedirect.com/science/article/pii/S0308814613015793
[34] Wang Z, Hu S, Bao H, Xing K, Liu J, Xia J, et al. Immunochromatographic assay based on time-resolved fluorescent nanobeads for the rapid detection of sulfamethazine in egg, honey, and pork. J Sci Food Agric [Internet]. 2021 Jan 30;101(2):684–92. Available from: https://doi.org/10.1002/jsfa.10681
[35] Pan M, Ma T, Yang J, Li S, Liu S, Wang S. Development of Lateral Flow Immunochromatographic Assays Using Colloidal Au Sphere and Nanorods as Signal Marker for the Determination of Zearalenone in Cereals. Foods [Internet]. 2020;9(3). Available from: https://www.mdpi.com/2304-8158/9/3/281
[36] Zhuang L, Gong J, Ji Y, Tian P, Kong F, Bai H, et al. Lateral flow fluorescent immunoassay based on isothermal amplification for rapid quantitative detection of Salmonella spp. Analyst [Internet]. 2020;145(6):2367–77. Available from: http://dx.doi.org/10.1039/C9AN02011J
[37] Cummins BM, Ligler FS, Walker GM. Point-of-care diagnostics for niche applications. Biotechnol Adv [Internet]. 2016;34(3):161–76. Available from: https://www.sciencedirect.com/science/article/pii/S0734975016300052
[38] Taylor LH, Latham SM, woolhouse MEJ. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci. 2001 Jul 29;356(1411):983–9.
[39] Wang H, Guan J, Liu X, Shi Y, Wu Q, Luo M, et al. Rapid detection of avian leukosis virus using a fluorescent microsphere immunochromatographic test strip assay. Poult Sci [Internet]. 2019;98(12):6492–6. Available from: https://www.sciencedirect.com/science/article/pii/S0032579119579561
[40] Manasa M, Revathi P, Chand MP, Maroudam V, Navaneetha P, Raj GD, et al. Protein-G-based lateral flow assay for rapid serodiagnosis of brucellosis in domesticated animals. J Immunoassay Immunochem [Internet]. 2019 Mar 4;40(2):149–58. Available from: https://doi.org/10.1080/15321819.2018.1541803
[41] Syahruni S, Hartati YW, Yusuf M, Kusumawardani S, Wibawan IWT, Arnafia W, et al. Development of lateral flow assay based on anti-IBDV IgY for the rapid detection of Gumboro disease in poultry. J Virol Methods [Internet]. 2021;291:114065. Available from: https://www.sciencedirect.com/science/article/pii/S0166093421000045
[42] Kumar R, Yadav SC, Kumar S, Dilbaghi N. Development of membrane-based flow-through assay for detection of trypanosomosis in equines. Journal of Parasitic Diseases [Internet]. 2020;44(1):99–104. Available from: https://doi.org/10.1007/s12639-019-01166-8
[43] Oertly M, Gerber V, Anhold H, Chan DS, Pusterla N. The Accuracy of Serum Amyloid A in Determining Early Inflammation in Horses After Long-Distance Transportation by Air. J Equine Vet Sci [Internet]. 2021;97:103337. Available from: https://www.sciencedirect.com/science/article/pii/S0737080620304287
[44] Masello M, Lu Z, Erickson D, Gavalchin J, Giordano JO. A lateral flow-based portable platform for determination of reproductive status of cattle. J Dairy Sci [Internet]. 2020;103(5):4743–53. Available from: https://www.sciencedirect.com/science/article/pii/S0022030220302095
[45] Garcia VS, Guerrero SA, Gugliotta LM, Gonzalez VDG. A lateral flow immunoassay based on colored latex particles for detection of canine visceral leishmaniasis. Acta Trop [Internet]. 2020;212:105643. Available from: https://www.sciencedirect.com/science/article/pii/S0001706X20300243
[46] Xu F, Jin Z, Zou S, Chen C, Song Q, Deng S, et al. EuNPs-mAb fluorescent probe based immunochromatographic strip for rapid and sensitive detection of porcine epidemic diarrhea virus. Talanta [Internet]. 2020;214:120865. Available from: https://www.sciencedirect.com/science/article/pii/S0039914020301569
[47] Chapman J, Truong VK, Elbourne A, Gangadoo S, Cheeseman S, Rajapaksha P, et al. Combining Chemometrics and Sensors: Toward New Applications in Monitoring and Environmental Analysis. Chem Rev [Internet]. 2020 Jul 8;120(13):6048–69. Available from: https://doi.org/10.1021/acs.chemrev.9b00616
[48] Almeida MIGS, Jayawardane BM, Kolev SD, McKelvie ID. Developments of microfluidic paper-based analytical devices (μPADs) for water analysis: A review. Talanta [Internet]. 2018;177:176–90. Available from: https://www.sciencedirect.com/science/article/pii/S0039914017309013
[49] Daverey A, Dutta K, Sarkar A. Chapter 1 - An overview of analytical methodologies for environmental monitoring. In: Kaur Brar S, Hegde K, Pachapur VL, editors. Tools, Techniques and Protocols for Monitoring Environmental Contaminants [Internet]. Elsevier; 2019. p. 3–17. Available from: https://www.sciencedirect.com/science/article/pii/B9780128146798000017

[50] Geissen V, Mol H, Klumpp E, Umlauf G, Nadal M, van der Ploeg M, et al. Emerging pollutants in the environment: A challenge for water resource management. International Soil and Water Conservation Research [Internet]. 2015;3(1):57–65. Available from: https://www.sciencedirect.com/science/article/pii/S2095633915000039
[51] Sollweck K, Streich P, Elsner M, Seidel M. A Chip-Based Colony Fusion Recombinase Polymerase Amplification Assay for Monitoring of Antimicrobial Resistance Genes and Their Carrying Species in Surface Water. ACS ES&T Water [Internet]. 2021 Mar 12;1(3):584–94. Available from: https://doi.org/10.1021/acsestwater.0c00110
[52] Marquez S, Liu J, Morales-Narváez E. Paper-based analytical devices in environmental applications and their integration with portable technologies. Curr Opin Environ Sci Health [Internet]. 2019;10:1–8. Available from: https://www.sciencedirect.com/science/article/pii/S2468584418300746
[53] Liu B, Zhuang J, Wei G. Recent advances in the design of colorimetric sensors for environmental monitoring. Vol. 7, Environmental Science: Nano. Royal Society of Chemistry; 2020. p. 2195–213.
[54] Lan J, Sun W, Chen L, Zhou H, Fan Y, Diao X, et al. Simultaneous and rapid detection of carbofuran and 3-hydroxy-carbofuran in water samples and pesticide preparations using lateral-flow immunochromatographic assay. Food Agric Immunol [Internet]. 2020 Jan 1;31(1):165–75. Available from: https://doi.org/10.1080/09540105.2019.1708272
[55] Li Y, Liu L, Kuang H, Xu C. Preparing monoclonal antibodies and developing immunochromatographic strips for paraquat determination in water. Food Chem [Internet]. 2020;311:125897. Available from: https://www.sciencedirect.com/science/article/pii/S0308814619320357
[56] Rames EK, Macdonald J. Rapid assessment of viral water quality using a novel recombinase polymerase amplification test for human adenovirus. Appl Microbiol Biotechnol [Internet]. 2019;103(19):8115–25. Available from: https://doi.org/10.1007/s00253-019-10077-w
[57] Berlina AN, Komova NS, Zherdev A V, Dzantiev BB. Combination of phenylboronic acid and oligocytosine for selective and specific detection of lead(ii) by lateral flow test strip. Anal Chim Acta [Internet]. 2021;1155:338318. Available from: https://www.sciencedirect.com/science/article/pii/S0003267021001446
[58] Hassan AHA, Bergua JF, Morales-Narváez E, Mekoçi A. Validity of a single antibody-based lateral flow immunoassay depending on graphene oxide for highly sensitive determination of E. coli O157:H7 in minced beef and river water. Food Chem [Internet]. 2019;297:124965. Available from: https://www.sciencedirect.com/science/article/pii/S0308814619310672
[59] Dzantiev BB, Taranova NA, Semeykina AA, Zherdev A V. Lateral flow immunoassay for bisphenol A: Development of test strips and their application for ecological monitoring. J Phys Conf Ser [Internet]. 2019;1172(1):012088. Available from: https://dx.doi.org/10.1088/1742-6596/1172/1/012088
[60] Zvereva EA, Hendrickson OD, Zherdev A V, Dzantiev BB. Immunochromatographic tests for the detection of microcystin-LR toxin in water and fish samples. Analytical Methods [Internet]. 2020;12(3):392–400. Available from: http://dx.doi.org/10.1039/C9AY01970G
[61] Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, et al. Solutions for a cultivated planet. Nature [Internet]. 2011;478(7369):337–42. Available from: https://doi.org/10.1038/nature10452
[62] Okawa K. Market and Trade Impacts of Food Loss and Waste Reduction. 2015; Available from: http://dx.doi.org/10.1787/5js4w29h0wr2-en
[63] Charkowski AO. The Changing Face of Bacterial Soft-Rot Diseases. Annu Rev Phytopathol [Internet]. 2018 Aug 25;56(1):269–88. Available from: https://doi.org/10.1146/annurev-phyto-080417-045906
[64] MANSFIELD J, GENIN S, MAGORI S, CITOVSKY V, SRIARIYANUM M, RONALD P, et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol [Internet]. 2012 Aug 1;13(6):614–29. Available from: https://doi.org/10.1111/j.1364-3703.2012.00804.x
[65] Chakraborty S. Migrate or evolve: options for plant pathogens under climate change. Glob Chang Biol [Internet]. 2013 Jul 1;19(7):1985–2000. Available from: https://doi.org/10.1111/gcb.12205
[66] DEAN R, VAN KAN JANAL, PRETORIUS ZA, HAMMOND-KOSACK KIME, DI PIETRO A, SPANU PD, et al. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol [Internet]. 2012 May 1;13(4):414–30. Available from: https://doi.org/10.1111/j.1364-3703.2011.00783.x
[67] MANSFIELD J, GENIN S, MAGORI S, CITOVSKY V, SRIARIYANUM M, RONALD P, et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol [Internet]. 2012 Aug 1;13(6):614–29. Available from: https://doi.org/10.1111/j.1364-3703.2012.00804.x
[68] Rybicki EP. A Top Ten list for economically important plant viruses. Arch Virol [Internet]. 2015;160(1):17–20. Available from: https://doi.org/10.1007/s00705-014-2295-9

[69] SCHOLTHOF KBG, ADKINS S, CZOSNEK H, PALUKAITIS P, JACQUOT E, HOHN T, et al. Top 10 plant viruses in molecular plant pathology. Mol Plant Pathol [Internet]. 2011 Dec 1;12(9):938–54. Available from: https://doi.org/10.1111/j.1364-3703.2011.00752.x
[70] Minsavage G V, Thompson CM, Hopkins DL, Leite R, Stall RE. Development of a polymerase chain reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology. 1994;84(5):456–61.
[71] Li W, Abad JA, French-Monar RD, Rascoe J, Wen A, Gudmestad NC, et al. Multiplex real-time PCR for detection, identification and quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip. J Microbiol Methods [Internet]. 2009;78(1):59–65. Available from: https://www.sciencedirect.com/science/article/pii/S0167701209001171
[72] Gutiérrez-Aguirre I, Mehle N, Delić D, Gruden K, Mumford R, Ravnikar M. Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. J Virol Methods [Internet]. 2009;162(1):46–55. Available from: https://www.sciencedirect.com/science/article/pii/S0166093409003358
[73] Haq MA, Collin HA, Tomsett AB, Jones MG. Detection of Sclerotium cepivorum within onion plants using PCR primers. Physiol Mol Plant Pathol [Internet]. 2003;62(3):185–9. Available from: https://www.sciencedirect.com/science/article/pii/S0885576503000237
[74] Wu J, Meng C, Shang H, Rong S, Zhang C, Hong J, et al. Monoclonal antibody-based triple antibody sandwich-enzyme-linked immunosorbent assay and immunocapture reverse transcription-polymerase chain reaction for Odontoglossum ringspot virus detection. J Virol Methods [Internet]. 2011;171(1):40–5. Available from: https://www.sciencedirect.com/science/article/pii/S0166093410003526
[75] Gorris MT, Sanz A, Peñalver J, López MM, Colomer M, Marco-Noales E. Detection and Diagnosis of Xylella fastidiosa by Specific Monoclonal Antibodies. Agronomy [Internet]. 2021;11(1). Available from: https://www.mdpi.com/2073-4395/11/1/48
[76] Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J, et al. Methods in virus diagnostics: From ELISA to next generation sequencing. Virus Res [Internet]. 2014;186:20–31. Available from: https://www.sciencedirect.com/science/article/pii/S016817021300436X
[77] Choi JR, Tang R, Wang S, Wan Abas WAB, Pingguan-Murphy B, Xu F. Paper-based sample-to-answer molecular diagnostic platform for point-of-care diagnostics. Biosens Bioelectron [Internet]. 2015;74:427–39. Available from: https://www.sciencedirect.com/science/article/pii/S0956566315302384
[78] Tang R, Yang H, Gong Y, You M, Liu Z, Choi JR, et al. A fully disposable and integrated paper-based device for nucleic acid extraction, amplification and detection. Lab Chip [Internet]. 2017;17(7):1270–9. Available from: http://dx.doi.org/10.1039/C6LC01586G
[79] Zhu G, Yin X, Jin D, Zhang B, Gu Y, An Y. Paper-based immunosensors: Current trends in the types and applied detection techniques. TrAC Trends in Analytical Chemistry [Internet]. 2019;111:100–17. Available from: https://www.sciencedirect.com/science/article/pii/S0165993618303881
[80] Khater M, de la Escosura-Muñiz A, Merkoçi A. Biosensors for plant pathogen detection. Biosens Bioelectron [Internet]. 2017;93:72–86. Available from: https://www.sciencedirect.com/science/article/pii/S0956566316309782
[81] Hodgetts J, Karamura G, Johnson G, Hall J, Perkins K, Beed F, et al. Development of a lateral flow device for in-field detection and evaluation of PCR-based diagnostic methods for Xanthomonas campestris pv. musacearum, the causal agent of banana xanthomonas wilt. Plant Pathol [Internet]. 2015 Jun 1;64(3):559–67. Available from: https://doi.org/10.1111/ppa.12289
[82] López-Soriano P, Noguera P, Gorris MT, Puchades R, Maquieira Á, Marco-Noales E, et al. Lateral flow immunoassay for on-site detection of Xanthomonas arboricola pv. pruni in symptomatic field samples. PLoS One [Internet]. 2017 Apr 27;12(4):e0176201-. Available from: https://doi.org/10.1371/journal.pone.0176201
[83] Scortichini M. Diagnostic protocols for regulated pests. Xylella fastidiosa. 2004;
[84] Selvarajan R, Kanichelvam PS, Balasubramanian V, Sethurama Subramanian S. A rapid and sensitive lateral flow immunoassay (LFIA) test for the on-site detection of banana bract mosaic virus in banana plants. J Virol Methods [Internet]. 2020;284:113929. Available from: https://www.sciencedirect.com/science/article/pii/S0166093420301816
[85] Ghosh DK, Kokane SB, Gowda S. Development of a reverse transcription recombinase polymerase based isothermal amplification coupled with lateral flow immunochromatographic assay (CTV-RT-RPA-LFICA) for rapid detection of Citrus tristeza virus. Sci Rep [Internet]. 2020;10(1):20593. Available from: https://doi.org/10.1038/s41598-020-77692-w
[86] Razo SC, Safenkova I V, Drenova N V, Kharchenko AA, Tsymbal YS, Varitsev YA, et al. New lateral flow immunoassay for on-site detection of Erwinia amylovora and its application on various organs of infected

plants. Physiol Mol Plant Pathol [Internet]. 2021;114:101637. Available from: https://www.sciencedirect.com/science/article/pii/S0885576521000382

[87] Ivanov A V, Shmyglya I V, Zherdev A V, Dzantiev BB, Safenkova I V. The Challenge for Rapid Detection of High-Structured Circular RNA: Assay of Potato Spindle Tuber Viroid Based on Recombinase Polymerase Amplification and Lateral Flow Tests. Plants [Internet]. 2020;9(10). Available from: https://www.mdpi.com/2223-7747/9/10/1369

[88] Ivanov A V, Safenkova I V, Drenova N V, Zherdev A V, Dzantiev BB. Development of lateral flow assay combined with recombinase polymerase amplification for highly sensitive detection of Dickeya solani. Mol Cell Probes [Internet]. 2020;53:101622. Available from: https://www.sciencedirect.com/science/article/pii/S0890850820301766

[89] Kettler H, White K, Hawkes SJ, Diseases UBSP for R and T in T. Mapping the landscape of diagnostics for sexually transmitted infections : key findings and recommendations / Hannah Kettler, Karen White, Sarah Hawkes [Internet]. Geneva: World Health Organization; 2004. Available from: https://apps.who.int/iris/handle/10665/68990

[90] Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. Journal of Antimicrobial Chemotherapy [Internet]. 2018 Apr 1;73(4):909–15. Available from: https://doi.org/10.1093/jac/dkx521

