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

**Kritika Shukla, Nikita, Md Salik Noorani\***

**Plant Molecular Virology Lab, Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi-110062, India.**

**Author’s detail**

*Kritika Shukla*

Plant Molecular Virology Lab

Department of Botany

School of Chemical and Life Sciences

Jamia Hamdard, New Delhi-110062, India.

*Nikita*

Plant Molecular Virology Lab

Department of Botany

School of Chemical and Life Sciences

Jamia Hamdard, New Delhi-110062, India.

**\****Md Salik Noorani*

Plant Molecular Virology Lab

Department of Botany

School of Chemical and Life Sciences

Jamia Hamdard, New Delhi-110062, India.

*Email: saliknoorani@jamiahamdard.ac.in*

**Abstract**

Lateral Flow Immunoassays (LFIA) have emerged as a 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. LFIA utilizes antibodies or nucleic acids as recognition elements, enabling qualitative and quantitative analysis. Its versatile application extends beyond clinical purposes to areas like food safety, veterinary medicine, environmental control, and agriculture. LFIA's simple strip-based design, combined with the benefits of chromatography and biorecognition probes, makes it an efficient and cost-effective analytical platform. The global market for lateral flow tests is projected to grow significantly, signifying its commercial success. Amidst the COVID-19 pandemic, LFIA has showcased its critical role in managing disease outbreaks. With the potential for rapid, specific, and sensitive on-site detection, LFIA paves the way for transformative advancements in the field of diagnostics.

1. **Introduction**

Conventional laboratory-based analytical techniques, including High-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA), and real-time polymerase chain reaction (qPCR), are known for their lengthy and complex procedures to obtain results (Campbell et al., 2020; Charlermroj et al., 2021). However, the demand for fast and on-site detection has prompted scientific research to focus increasingly on the development and enhancement of portable, cost-effective, and user-friendly quick analysis techniques for point-of-care (POC) testing (Hansen & Abd El Wahed, 2020; Soh et al., 2020).

In response to this need, point-of-care devices based on lateral flow assays (LFA) have emerged as a rapidly expanding method for qualitative and quantitative analysis. LFAs can be categorized into two types based on the elements of recognition used: Lateral flow immunoassay (LFIA) that employs antibodies as exclusive recognition elements and nucleic acid lateral flow assay (NALFA) where recognition elements include nucleic acids.

Among these methods, the Lateral Flow Immunoassay (LFIA) has proven to be one of the most effective analytical platforms for on-site detection of target analytes. LFIA is akin to a lab-in-a-hand, designed to accelerate decision making and turnaround time. Its application has rapidly expanded beyond clinical purposes to other domains such as food and feed safety, veterinary medicine, environmental control, agriculture, and more, detecting molecules, organisms, and (bio) markers.

The LFIA procedure involves a strip with several components on a plastic background, including the nitrocellulose membrane, adsorption pad, conjugate pad, and sample application pad. Test and control lines are present in the nitrocellulose membrane. Upon the flow of the liquid sample, pre-immobilized reagents at various locations along the strip become active, combining the advantages of chromatography and biorecognition probes (Sajid et al., 2015).

LFA strips offer numerous benefits, including rapidity and one-step analysis, low operational cost, simple instrumentation, user-friendly format, higher specificity, better sensitivity, long-term stability under various environmental conditions, and portability of the device (Li et al., 2010).

In the commercial environment, the success of LFIA testing is evident, with the global market for lateral flow tests expected to reach $10.36 billion in US sales by 2027, growing at a compound annual growth rate (CAGR) of 7.7 percent from 2020 to 2027. In 2019, the market was already valued at approximately $5.98 billion in US dollars (1).

Undoubtedly, the significance of LFIA testing is underscored by its application in managing the global coronavirus disease (COVID-19) pandemic caused by the novel coronavirus (SARS-CoV-2). The versatility and efficiency of LFIA have made it an essential tool in various fields, demonstrating its immense potential for on-site detection and diagnostics.

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

|  |  |
| --- | --- |
| Advantages | Disadvantages |
| 1. Light-weight & portable.
2. Less analysis time.
3. Low cost.
4. Several applications.
5. Small sample volume is required.
6. Simple and user-friendly operation.
7. Very long shelf life and stable over a wide range of environmental conditions.
8. Comparable or better sensitivity and specificity than other well-known techniques.
9. High commercialization potential.
10. Easy integration with electronics.
11. Versatility of formats, bio-recognition molecules, labels and detection systems.
12. Energy consumption is nil or very low.
 | 1. Mostly qualitative or semi-quantitative.
2. Subjective result interpretation.
3. Technological improvements usually increase the cost per analysis.
4. Sometimes, pretreatment of sample is required which is time consuming.
5. Poor affinity of biomolecules for analytes and a susceptibility for cross-reactivity.
6. Analysis time is also dependent on nature of sample itself i.e., viscosity, surface tension.
7. Once sample is applied to the strip, capillary action cannot be decreased or speeded up.
 |

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 (Pai et al., 2012; Parolo & Merkoçi, 2013; Soh et al., 2020). 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 (Pai et al., 2012; Soh et al., 2020). LFIAs are inherently suitable for application in settings other than laboratories (Pai et al., 2012). 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 (Pai et al., 2012; Soh et al., 2020; Urusov et al., 2019). The pregnancy test is without a doubt the test that represents clinical diagnostics the best (Urusov et al., 2019; Weihs et al., 2021). However, other additional LFIAs have been created and applied over time for various clinical objectives.

1. Foodborne pathogens and toxins

Food quality is impacted at every stage, from transportation to processing (Galindo et al., 2014; Utrera et al., 2014). 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 (Gracias & McKillip, 2004). 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). 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 (Nuntawong et al., 2022). 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. 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). 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 (Ching et al., 2012). 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 (Wang et al., 2013) 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) (Fang et al., 2014). For the purpose of detecting Vibrio cholera, freshly formed antibodies in combination with AuNPs were used in LFA (Chen et al., 2014). 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 (C.-C. Liu et al., 2013). 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 (Wiriyachaiporn et al., 2013). 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 (Song et al., 2013). 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 (Koizumi et al., 2014).

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. (Cummins et al., 2016).

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 (Chapman et al., 2020). 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 (Almeida et al., 2018). 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, labor-intensive, and frequently time-consuming. Environmental analyses involve extensive knowledge of advanced analytical chemistry, as well as sophisticated and costly apparatus (Chapman et al., 2020). Pathogens are typically identified using polymerase chain reaction (PCR)-based detection, while pollutants are detected in the lab using chromatographic and spectroscopic approaches (Marquez et al., 2019). 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 (B. Liu et al., 2020). 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).

1. Agriculture
2. **Conclusion**

In conclusion, the increasing demand for prompt and efficient on-site diagnostic techniques has driven the emergence of point-of-care (POC) testing in various fields like clinical analysis, food safety, agriculture, and environmental analysis. Lateral flow immunoassay (LFIA), also known as immunochromatographic strip test or rapid diagnostic test, stands out as one of the most effective analytical platforms for decentralized testing, offering quick and reliable results without the need for complex infrastructure. LFIA fulfills the criteria of an ideal POCT, being Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Delivered (ASSURED). It eliminates the need for trained personnel, pipetting, washing, and cold chain requirements. Gold or silver nanoparticles are commonly used as labels for secondary antibodies in LFIA, allowing the identification of pathogen-specific antigens and/or antibodies within 10 to 30 minutes. The recent development of multiplex LFIA enables the detection of multiple targets in a single test, further enhancing its potential for transformative advancements in diagnostics

**References**

Almeida, M. I. G. S., Jayawardane, B. M., Kolev, S. D., & McKelvie, I. D. (2018). Developments of microfluidic paper-based analytical devices (μPADs) for water analysis: A review. Talanta, 177, 176–190. https://doi.org/https://doi.org/10.1016/j.talanta.2017.08.072

Boutal, H., Vogel, A., Bernabeu, S., Devilliers, K., Creton, E., Cotellon, G., Plaisance, M., Oueslati, S., Dortet, L., Jousset, A., Simon, S., Naas, T., & Volland, H. (2018). 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, 73(4), 909–915. https://doi.org/10.1093/jac/dkx521

Campbell, V. R., Carson, M. S., Lao, A., Maran, K., Yang, E. J., & Kamei, D. T. (2020). Point-of-Need Diagnostics for Foodborne Pathogen Screening. SLAS TECHNOLOGY: Translating Life Sciences Innovation, 26(1), 55–79. https://doi.org/10.1177/2472630320962003

Chapman, J., Truong, V. K., Elbourne, A., Gangadoo, S., Cheeseman, S., Rajapaksha, P., Latham, K., Crawford, R. J., & Cozzolino, D. (2020). Combining Chemometrics and Sensors: Toward New Applications in Monitoring and Environmental Analysis. Chemical Reviews, 120(13), 6048–6069. https://doi.org/10.1021/acs.chemrev.9b00616

Charlermroj, R., Phuengwas, S., Makornwattana, M., Sooksimuang, T., Sahasithiwat, S., Panchan, W., Sukbangnop, W., Elliott, C. T., & Karoonuthaisiri, N. (2021). Development of a microarray lateral flow strip test using a luminescent organic compound for multiplex detection of five mycotoxins. Talanta, 233, 122540. https://doi.org/https://doi.org/10.1016/j.talanta.2021.122540

Chen, W., Zhang, J., Lu, G., Yuan, Z., Wu, Q., Li, J., Xu, G., He, A., Zheng, J., & Zhang, J. (2014). Development of an immunochromatographic lateral flow device for rapid diagnosis of Vibrio cholerae O1 serotype Ogawa. Clinical Biochemistry, 47(6), 448–454. https://doi.org/https://doi.org/10.1016/j.clinbiochem.2013.12.022

Ching, K. H., Lin, A., McGarvey, J. A., Stanker, L. H., & Hnasko, R. (2012). Rapid and selective detection of botulinum neurotoxin serotype-A and -B with a single immunochromatographic test strip. Journal of Immunological Methods, 380(1), 23–29. https://doi.org/https://doi.org/10.1016/j.jim.2012.03.008

Cummins, B. M., Ligler, F. S., & Walker, G. M. (2016). Point-of-care diagnostics for niche applications. Biotechnology Advances, 34(3), 161–176. https://doi.org/https://doi.org/10.1016/j.biotechadv.2016.01.005

Fang, Z., Wu, W., Lu, X., & Zeng, L. (2014). Lateral flow biosensor for DNA extraction-free detection of salmonella based on aptamer mediated strand displacement amplification. Biosensors and Bioelectronics, 56, 192–197. https://doi.org/https://doi.org/10.1016/j.bios.2014.01.015

Galindo, A., Calín-Sánchez, Á., Collado-González, J., Ondoño, S., Hernández, F., Torrecillas, A., & Carbonell-Barrachina, Á. A. (2014). Phytochemical and quality attributes of pomegranate fruits for juice consumption as affected by ripening stage and deficit irrigation. Journal of the Science of Food and Agriculture, 94(11), 2259–2265. https://doi.org/https://doi.org/10.1002/jsfa.6551

Gracias, K. S., & McKillip, J. L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. Canadian Journal of Microbiology, 50(11), 883–890. https://doi.org/10.1139/w04-080

Hansen, S., & Abd El Wahed, A. (2020). Point-Of-Care or Point-Of-Need Diagnostic Tests: Time to Change Outbreak Investigation and Pathogen Detection. Tropical Medicine and Infectious Disease, 5(4). https://doi.org/10.3390/tropicalmed5040151

Kettler, H., White, K., Hawkes, S. J., & Diseases, U. B. S. P. for R. and T. in T. (2004). Mapping the landscape of diagnostics for sexually transmitted infections : key findings and recommendations / Hannah Kettler, Karen White, Sarah Hawkes. World Health Organization. https://apps.who.int/iris/handle/10665/68990

Koizumi, D., Shirota, K., Akita, R., Oda, H., & Akiyama, H. (2014). Development and validation of a lateral flow assay for the detection of crustacean protein in processed foods. Food Chemistry, 150, 348–352. https://doi.org/https://doi.org/10.1016/j.foodchem.2013.10.130

Li, Z., Wang, Y., Wang, J., Tang, Z., Pounds, J. G., & Lin, Y. (2010). Rapid and Sensitive Detection of Protein Biomarker Using a Portable Fluorescence Biosensor Based on Quantum Dots and a Lateral Flow Test Strip. Analytical Chemistry, 82(16), 7008–7014. https://doi.org/10.1021/ac101405a

Liu, B., Zhuang, J., & Wei, G. (2020). Recent advances in the design of colorimetric sensors for environmental monitoring. In Environmental Science: Nano (Vol. 7, Issue 8, pp. 2195–2213). Royal Society of Chemistry. https://doi.org/10.1039/d0en00449a

Liu, C.-C., Yeung, C.-Y., Chen, P.-H., Yeh, M.-K., & Hou, S.-Y. (2013). Salmonella detection using 16S ribosomal DNA/RNA probe-gold nanoparticles and lateral flow immunoassay. Food Chemistry, 141(3), 2526–2532. https://doi.org/https://doi.org/10.1016/j.foodchem.2013.05.089

Marquez, S., Liu, J., & Morales-Narváez, E. (2019). Paper-based analytical devices in environmental applications and their integration with portable technologies. Current Opinion in Environmental Science & Health, 10, 1–8. https://doi.org/https://doi.org/10.1016/j.coesh.2019.08.002

Nuntawong, P., Putalun, W., Tanaka, H., Morimoto, S., & Sakamoto, S. (2022). Lateral flow immunoassay for small-molecules detection in phytoproducts: a review. Journal of Natural Medicines, 76(3), 521–545. https://doi.org/10.1007/s11418-022-01605-6

Pai, N. P., Vadnais, C., Denkinger, C., Engel, N., & Pai, M. (2012). Point-of-Care Testing for Infectious Diseases: Diversity, Complexity, and Barriers in Low- And Middle-Income Countries. PLOS Medicine, 9(9), e1001306-. https://doi.org/10.1371/journal.pmed.1001306

Parolo, C., & Merkoçi, A. (2013). Paper-based nanobiosensors for diagnostics. Chem. Soc. Rev., 42(2), 450–457. https://doi.org/10.1039/C2CS35255A

Parolo, C., Sena-Torralba, A., Bergua, J. F., Calucho, E., Fuentes-Chust, C., Hu, L., Rivas, L., Álvarez-Diduk, R., Nguyen, E. P., Cinti, S., Quesada-González, D., & Merkoçi, A. (2020). Tutorial: design and fabrication of nanoparticle-based lateral-flow immunoassays. Nature Protocols, 15(12), 3788–3816. https://doi.org/10.1038/s41596-020-0357-x

Sajid, M., Kawde, A.-N., & Daud, M. (2015). Designs, formats and applications of lateral flow assay: A literature review. Journal of Saudi Chemical Society, 19(6), 689–705. https://doi.org/https://doi.org/10.1016/j.jscs.2014.09.001

Soh, J. H., Chan, H.-M., & Ying, J. Y. (2020). Strategies for developing sensitive and specific nanoparticle-based lateral flow assays as point-of-care diagnostic device. Nano Today, 30, 100831. https://doi.org/https://doi.org/10.1016/j.nantod.2019.100831

Song, C., Zhi, A., Liu, Q., Yang, J., Jia, G., Shervin, J., Tang, L., Hu, X., Deng, R., Xu, C., & Zhang, G. (2013). Rapid and sensitive detection of β-agonists using a portable fluorescence biosensor based on fluorescent nanosilica and a lateral flow test strip. Biosensors and Bioelectronics, 50, 62–65. https://doi.org/https://doi.org/10.1016/j.bios.2013.06.022

Urusov, A. E., Zherdev, A. V, & Dzantiev, B. B. (2019). Towards Lateral Flow Quantitative Assays: Detection Approaches. Biosensors, 9(3). https://doi.org/10.3390/bios9030089

Utrera, M., Morcuende, D., & Estévez, M. (2014). Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. LWT - Food Science and Technology, 56(1), 62–68. https://doi.org/https://doi.org/10.1016/j.lwt.2013.10.040

Wang, Y.-K., Shi, Y.-B., Zou, Q., Sun, J.-H., Chen, Z.-F., Wang, H., Li, S.-Q., & Yan, Y.-X. (2013). Development of a rapid and simultaneous immunochromatographic assay for the determination of zearalenone and fumonisin B1 in corn, wheat and feedstuff samples. Food Control, 31(1), 180–188. https://doi.org/https://doi.org/10.1016/j.foodcont.2012.09.048

Weihs, F., Anderson, A., Trowell, S., & Caron, K. (2021). Resonance Energy Transfer-Based Biosensors for Point-of-Need Diagnosis—Progress and Perspectives. Sensors, 21(2). https://doi.org/10.3390/s21020660

Wiriyachaiporn, S., Howarth, P. H., Bruce, K. D., & Dailey, L. A. (2013). Evaluation of a rapid lateral flow immunoassay for Staphylococcus aureus detection in respiratory samples. Diagnostic Microbiology and Infectious Disease, 75(1), 28–36. https://doi.org/https://doi.org/10.1016/j.diagmicrobio.2012.09.011

Global Lateral Flow Assay Market Size by Type, by Technique, by Application, by End-user, by Geography and Forecast. [(accessed on 17 May 2021)]; Available online: https://www.verifiedmarketresearch.com/product/lateral-flow-assay-market/

WHO. 2022. Food safety. https://www.who.int/news-room/fact-sheets/detail/food-safety.

Amiard J.-C., Amiard-Triquet C. Quality Standard Setting and Environmental Monitoring. In: Amiard-Triquet C., Amiard J.-C., Moneyrac C., editors. Aquatic Ecotoxicology. 1st ed. Academic Press; Cambridge, MA, USA: 2015. pp. 51–76.

WHO WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007–2015. [(accessed on 17 May 2021)]; Available online: http://apps.who.int/iris/bitstream/10665/199350/1/9789241565165\_eng.pdf