Sensors & Diagnostics

EDITORIAL

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Cite this: *Sens. Diagn.*, 2025, **4**, 721

DOI: 10.1039/d5sd90027a

rsc.li/sensors

Open Access Article. Published on 14 srpna 2025. Downloaded on 26.05.2026 12:33:25.
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Introduction to "Lateral Flow Assays: Methods and Applications"

Jing Wang, *[a] Jiangjiang Zhang [b] and Yanmin Ju [c]

We are delighted to welcome you to this innovative and timely themed collection, *Lateral Flow Assays: Methods and Applications*, which showcases cutting-edge research in the design, development, and deployment of lateral flow technologies. The contributions span a wide range of topics, including novel nanomaterials, signal amplification strategies, point-of-care diagnostics, and real-world applications in healthcare, food safety, and environmental monitoring.

In their Tutorial Review (**https://doi.org/10.1039/D4SD00206G**), Lu and Chan presented a study of antibody targeting in lateral flow immunoassay technology and focus on the impact of antibody targeting on sensitivity and specificity. They reviewed various modification techniques, including non-covalent modifications (protein A/G, biotin–streptavidin) and covalent modifications (amino–carboxyl, disulfide bonds, *etc.*), as well as modifications to test strip components (detection line, control line, nitrocellulose membrane). By maximizing the exposure of binding sites through targeted immobilization of antibodies, the detection limit can be significantly reduced and non-specific binding minimized. They further compared the advantages and disadvantages of different techniques, noting that covalent modifications offered high specificity but involved complex steps, while non-covalent methods faced challenges related to reversibility, and cellulose materials required solutions for issues such as fluorescence diffusion. This review provides theoretical and technical references for optimizing the performance of lateral flow immunoassay technology.

Acute promyelocytic leukemia (APL) is a malignant blood tumor characterized by severe complications and a high mortality rate. However, traditional diagnostic methods (such as karyotyping, fluorescence *in situ* hybridization, and reverse transcription polymerase chain reaction) are typically time-consuming and unable to meet the demands of emergency treatment. Chabi and colleagues developed a europium-based lateral flow immunoassay capable of directly detecting the PML-RARα fusion oncogene protein within the nucleus. In their article (**https://doi.org/10.1039/D4SD00357H**), they efficiently captured leukocytes from whole blood and lysed them to release nuclear proteins, with a total turnaround time of less than 30 minutes. They then used europium chelate nanoparticles as fluorescent reporter molecules, reducing background interference by delaying the detection of fluorescent signals. The technology demonstrated approximately 11% limit of detection for detecting NB4 cells mixed with normal peripheral blood mononuclear cells or human acute myeloid leukemia HL-60 cells, compressing APL diagnosis time from the "day-level" of traditional methods to the "hour-level," providing a critical early screening tool for emergency situations.

In their paper (**https://doi.org/10.1039/D4SD00342J**), O'Sullivan and co-authors present the development of a dual-aptamer-based lateral flow assay (ALFA) for the detection of *Trichomonas vaginalis*, offering a promising contribution to the WHO's global research priorities on sexually transmitted infections (STIs). This ALFA, notable for eliminating reliance on biotin/streptavidin systems, employs UV-crosslinked aminated capture aptamers and thioctic acid-modified aptamer–AuNP conjugates. Impressively, the test achieves a detection limit of 1.6×10^5 parasites per mL, demonstrates excellent specificity, and shows no matrix interference from clinical CVL samples. With a low production cost (<1 €), room-temperature stability, and simplified operation, this assay presents a practical and scalable POCT solution for STI diagnostics.

In their study, Lillehoj *et al.* address a critical bottleneck in CRISPR-Cas-based lateral flow assays (LFAs)—the subjectivity and potential inaccuracy of visual interpretation (**https://doi.org/10.1039/D4SD00314D**). By developing and validating two neural network models, including a lightweight mobile-optimized version, they offer an automated and highly accurate solution for real-time interpretation of CRISPR-Cas13-based LFAs targeting the SARS-CoV-2 N gene. Trained on over 3000 smartphone-captured images under diverse conditions, the models achieved

[a] *Zhejiang University of Technology, China. E-mail: jingw1986@zjut.edu.cn*
[b] *Beijing Institute of Technology, China*
[c] *China Pharmaceutical University, China*

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96.5% accuracy with rapid classification speeds (0.2 s). This work underscores the power of integrating machine learning with diagnostic LFAs to facilitate reliable self-testing and broader point-of-care deployment.

Complementary to the AuNPs-based naked eye recognition, the surface-enhanced Raman scattering (SERS) technique was introduced to improve the sensitivity of LFAs. Strobbia and Steckl's group report a SERS-enhanced LFA for oral health monitoring by detecting the biomarker lipopolysaccharides (LPS) from *P. gingivalis*. They used a SERS tag to screen AuNPs and Au or Ag@Au nanostars with tunable size and absorption properties for maximum enhancement. By using a portable Raman detector, they achieved a limit of detection (LOD) <10 ng mL^{-1}, over 10 times lower than the conventional colorimetric LFAs (**https://doi.org/10.1039/D4SD00056K**).

Traditional LFAs build on the principle of specific recognition between antigen and antibody, since the protein antibody displays the highest binding affinity to the antigen. However, the production of protein antibodies is costly and time-consuming. A DNA aptamer, a single-strand oligonucleotide with specific sequences showing high binding affinity, is a promising alternative to protein antibodies, and it is much cheaper than the protein antibody. Aiming for aflatoxin B1 detection, Ebanks' group discovered a new aptamer with a dissociation constant of ~42 nM and developed the aptamer-based LFA, where aflatoxin B1 and AuNPs competitively bind the aptamer resulting in the elimination of the test line color. They successfully tested the application in water and peanut samples (**https://doi.org/10.1039/D4SD00271G**).

Illegally recycled waste cooking oil, containing various carcinogens, is a big threat to human health. The identification of refined gutter oil relies on costly and complicated chromatographic or spectroscopic techniques. Shi's group explored a non-organic solvent extraction method to gather a key component of capsaicinoids in waste cooking oil, and developed a competitive fluorescent LFA for capsaicinoids detection and waste cooking oil identification. Within a total process time of 15 minutes, they achieved cheap, fast, and sensitive detection of natural capsaicin with a LOD of 0.14 μg kg^{-1}, fully meeting the critical standard value of 1.0 μg kg^{-1} ("Determination of Capsaicin in Edible Oils" – BJS201801, the China National Food and Drug Administration). Cross-reactivity rates for dihydrocapsaicin and synthetic capsaicin exceed 90% (**https://doi.org/10.1039/D4SD00306C**).

Taken together, this collection highlights the remarkable versatility and continuing evolution of lateral flow assays, with contributions from researchers across the globe. We hope that readers will be inspired by these representative studies to further explore the vast potential of lateral flow assays – from enhancing sensitivity and integrating digital readouts to broadening applications in medical diagnostics, environmental monitoring, and food safety.

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