SLOVAK MEDICAL UNIVERSITY IN BRATISLAVA
FACULTY OF MEDICINE
Institute of Chemistry, Clinical Biochemistry and Laboratory Medicine
Limbová 12, 833 03 Bratislava 37

LATERAL FLOW ASSAYS AND POINT-OF-CARE FOR BLOOD TESTING AND ITS PERSPECTIVE IN THE FUTURE

Author:
Alena Laura MERZ
General Medicine, 5 GM

Supervisor:
Ing. Lucia Hudecová
Institute of Chemistry, Clinical Biochemistry and Laboratory Medicine; Faculty of Medicine of Slovak Medical University; Limbová 12, 833 03 Bratislava 37

ABSTRACT

Point-of-care refers to testing at time and place of patient care without the usage of a medical laboratory. The demand for point-of-care multiple diagnostic assays allows the rapid detection of several analytes present in samples e.g. in urine, blood, saliva or other body fluids.

Lateral flow assays (LFAs) are a subcategory of point-of-care testing and progressively gain more popularity among detection tests. These tests are the technology of paper-based platform for the detection and quantification of analytes in mixtures popular in biomedicine and environmental sciences. LFA-based tests are widely used in hospitals, doctor's offices and laboratories for the quantitative and qualitative detection of specific antigens as well as antibodies.

This research targets to present an overview of the principle of the LFA method and the crucial components of the assay, focusing primarily on immunoassays as well as its perspective in the future.

Key words: lateral flow assays, point-of-care testing, immunoassays, quantitative and qualitative detection, antigens, antibodies.

ABSTRAKT

"Point-of-care" testy sú testy vykonávané v čase a na mieste poskytovania zdravotnej starostlivosti pacientovi bez použitia biomedicínskeho laboratória. Viacnásobné "point-of-care" diagnostické testy umožňujú naraz rýchlu detekciu niekoľkých analytov prítomných vo vzorkách, napr. vo vzorke moču, krvi, slinách alebo iných telesných tekutinách.

Laterálne prietokové testy (LFA) sú podkategóriou "point-of-care" testov a postupne získavajú väčšiu popularitu medzi detekčnými testami. Sú to testy technologicky založené na papierovej báze používané na detekciu a kvantifikáciu analytov zmesí stanovovaných v biomedicíne a environmentálnych vedách. Testy na báze LFA sa vo veľkej miere používajú v nemocniciach, ambulanciách a laboratóriách na kvantitatívne a kvalitatívne stanovenie špecifických antigénov, ako aj protilátok.

Cieľom tejto práce je podať prehľad o princípe metód LFA a kľúčových komponentoch týchto testov, so zameraním predovšetkým na testy na imunitu, ako aj na ich perspektívy do budúcnosti.

Kľúčové slová: laterálne prietokové testy, "point-of-care" testy, testy na imunitu, kvantitatívna a kvalitatívna detekcia, antigény, protilátky.

CONTENT

INTRODUCTION

Lateral flow assays (LFAs) belong to point-of-care (POC) diagnostics and are abundantly utilized as they possess various benefits [6]. They detect and quantify analytes in mixtures, where results are obvious within up to 30 minutes. Some of the samples that can be tested through lateral flow assays include blood, serum, plasma, saliva, cerebrospinal fluid, urine, feces, soil or food.

As being paper based, they demand low production costs and are easily modifiable, allowing them to be applicable to multiple fields. Furthermore, hospital and healthcare settings and medical laborites extensively use them as they are considered to be feasible and convenient. Also other institutions and facilities use LFAs e.g. environmental quality control, food industry or veterinary medicine.

LFAs are simply employed because they do not require laboratory settings or medical personnel, they can be stored in non-demanding settings and can be conducted within patients proximity. Multiple analytes and diagnostic parameters can be tested through the assays already, making them extremely useful and convenient.

Moreover, the current broad research and studies on LFAs, pose promising advances and developments in the future, constituting to POC testing being a burgeoning market worldwide.

POC (Point-of-care Testing)

Definition

Point-of-care testing is referred to as the analysis of specimens outside central clinical laboratories, ideally next to patients bed. This does not only apply for hospital settings, but POC tests can be easily conducted at home as well.

Examples include pregnancy testing, urine dipstick analysis or infectious agents detection, including HIV, MRSA or Influenzae.

Classification

According to the source, classification of POC tests vary slightly.

Overall, they can be distinguished into certain types depending on their discriminative value and complexity.

Qualitatively strip-based POCT methods work on differentiating between plus and minus results and rely on optical detection. From chemical indicator reaction (urine dipstick analysing glucose, bilirubin, ketone, blood, leucocytes etc.) to immunological reactions (hCG or infectious disease detection), they are extremely valuable.

Furthermore, unit-use analyzers, a different type of POC, operate on test strips which is read by a device, whereas, the reaction has already occurred. A very well-known example is the glucometer for blood glucose testing. Blood glucose monitoring is essential for patients who have diabetes or belong to its risk group, however, it has become a very pivotal tool in hospital settings allowing the observation of blood glucose in general and in response to medications or lifestyle changes. Pathological glycemic fluctuations may lead to severe consequences, such as organ damage, strokes or myocardial infarcts [8].

Another type of POC are the bench-top POCT analyzers, which are more sophisticated and utilize different principles. To these principles belong spectrophotometry, hematological multichannel analyzers, blood gas analyzers or immunological devices.

Other types involve hematostaseological coagulation analyzers and molecular biology based POCT (immunochromatography and DNA amplification) [7].

POC testing versus central laboratory testing

When comparing POC testing and central clinical laboratory testing, several advantages and disadvantages can be elucidated. Benefits of POC testing are e.g. the reduction of staff personnel and procedure steps, saving money and time as well faster results and treatment decisions. Also, in a hospital setting, the test results can immediately become integrated into the clinics technical systems improving record.

In central laboratory testing, complex and more test principles can be conducted and high volume specimens can be used. A POC test cannot handle high number of specimens and may not be as accurate and precise as central laboratory testing. However, also in central laboratories the process is complicated and costly equipment as well as qualified personnel are demanded, limiting the use of this type of testing.

POC testing suits best for bedside or personal testing with easy and cost-effective methods for quick and feasible results [5]. Only small sample volumes (up to 1 microliters) are needed, easing the comfort for the patient as normal venous blood collection requires higher amounts of blood and its peripheral procedure is longer and stressful.

Fewer steps are necessary for handling POC testing, which mitigates error in pre- and postanalytical processes compared to central laboratory testing [10].

It must be also understood that preanalytical errors can arise from poor sampling or inappropriate strip storage or concomitant medication use. Thus, healthcare personnel must be trained correctly and devices must regularly maintained to prevent inaccuracies [3].

LATERAL FLOW ASSAYS

Definition

Lateral flow assays are tests based on biochemical interaction of antigen-antibody or probe DNA-target DNA hybridization.

Classification of methods

Later flow assays can be classified as lateral flow immunoassays and nucleic acid lateral flow assays, depending on the elements that are involved [4].

Lateral flow immunoassay (LFI), or 'dipstick' assay or immunochromatographic assays, is designed to detect an antibody or antigen in the clinical sample. The samples include saliva, urine, plasma, serum or cerebrospinal fluid. A very well-known example of LFI is the urinary detection of human Chorionic Gonadotropin, or also known as a pregnancy test, which set a milestone in POC testing. Additionally, there are several varieties of immunoassays, primarily either detecting antibodies or other targets e.g. proteins or hormones. Examples include ELISA (enzyme-linked immunosorbent assay), immunocytochemistry, flow cytometry or immunohistochemistry, all pertinent to different analytes [1]. Lateral flow assays can also be distinguished into nucleic acid lateral flow assay which recognize nucleic acids as seen in the table (Table 1) below.

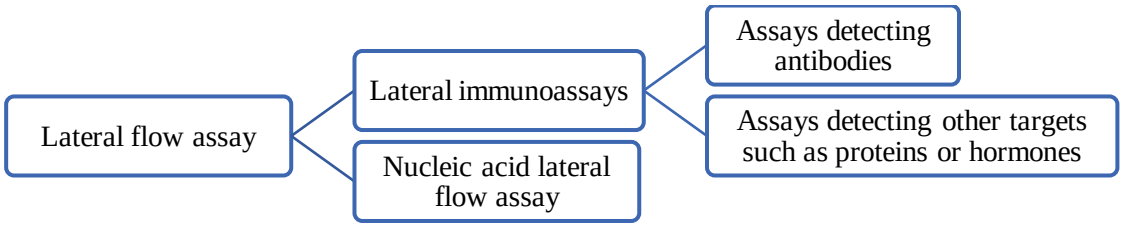

Table 1. Classification of lateral flow assays

Design and components of assays

A lateral flow immunoassay is a strip-like test, composed of a sample pad, conjugate release pad, detection zone and test/control line. The analyte migrates without capillary action and interacts with the attached molecules contributing to the readout.

The analyte, which is within the liquid sample, is placed on the low-protein binding, absorbent sample pad [4], which is typically composed of cellulose fiber. Cellulose fiber have a large volume bed and are inexpensive. The purpose of the pad is to evenly distribute the sample and ensuring the capability of binding to reagents on the conjugate pad [1].

The conjugate release pad contains particular antibodies and is conjugated to colored particles, which are often colloidal gold or latex. The release pad crucially controls the flow of the LFA and can be modified accordingly. The sample with the conjugated antibody which is bound to the analyte passes along the membrane strip until it reaches the detection zone which is impregnated with antibodies/antigens [4].

The membrane usually consists of nitrocellulose and is the most pivotal part of the assay.

The antibodies or antigens on the membrane are immobilized and react with the antibody-bound analyte thereby recognizing the analyte, which results in a response seen on the test line.

The test line binds the sample protein, whereas the control line contains the species-specific antibodies for the detection of the antibodies bound to the analyte. The control line indicates that the performance is valid and has been conducted appropriately.

Lastly, the wicking pad washes away non-interacted sample to counteract any backflow [1].

Immunoassays

There are direct and competitive lateral flow immunoassays.

The direct test is frequently utilized for larger analytes or analytes with various antigenic sites. Examples include HIV or pregnancy tests.

Smaller molecules with specific antigenic sites, or those who cannot simultaneously bind to two antibodies are categorized as competitive tests. The competitive test functions by the blockage of the binding sites of antibodies by analytes on the test line inhibiting any contact or reaction, therefore, a positive result is shown through the missing signal in the test line [4]. The intensity of the color at the test line can be seen as equal to the analyte present in the sample.

Within the same sample, multiple analytes can be identified, which increases the intricacy of a lateral flow immunoassay.

Regardless of the complexity, all lateral flow immunoassays display a formation complex between the detection reagent and a capturing reagent.

Contemplating the benefits of an lateral flow immunoassay, it is crucial to mention the inexpensive cost expenditures, its advanced technology, its abundancy in application as well as its low maintenance. Additionally, it can be integrated with reading and detection systems and is known to be feasible.

However, the readout remains semi-quantitively and the tested volume may limit overall sensitivity, thus decreasing accuracy [9].

Nucleic acid lateral flow assays are emerging and are based on immobilized DNA molecules at the surface of nitrocellulose membranes. They recognize specific DNA or RNA sequences, allergens, thrombin, salivary markers, toxins or pathogens as well as drugs or metallix ions. Such testing of nucleic acids is important in environmental or food monitoring, and cumulatively in medical diagnostics.

Additionally, they are rapid, inexpensive and easy to implement, making them quite beneficial and attractive.

Consequently, due to rapid developments, nucleic acid lateral flow assays can be distinguished into nucleic acid lateral flow and nucleic acid lateral flow immunoassay.

Nucleic acid lateral flow immunoassays operate through direct DNA exploiting capture detection and oligonucleotide probes whereas its approaching examples are far less common than nucleic acid lateral flow assays which use antibodies to detect hapten-labelled DNA [2].

New strategies in LFAs

Considering the prospective future of lateral flow assays, it is important to contemplate that a multidisciplinary approach must be done in order to keep up with the market. This includes labelling, handling of samples, integration of reading or exterior design.

Sample handling in correlation to sensitivity to assay must be properly integrated into the whole test and several factors must be considered to create a highly sensitive sample.

One of the includes that there must be an excess of antibodies (capture and label) to give accurate results, because only when antibodies are in excess, the dose-response curve will be positive.

Reading systems are also evolving as some of the visual labels of lateral flow immunoassays do not need readers, which minimizes costs. Reading system are particular the most expandable part of the assay and integrated readers are the driving force.

Moreover, the trend is towards home diagnostic tests which do not require complicated reading devices, but smart connections of data collection and its interpretation or storage.

There are also several novel labelling technologies, which are yet to be approved but pose promising innovations e.g. Up-converting Phosphor Technology and is based on ceramic particles containing Ianthanide, which can absorb infrared light and also emit light, having the advantage that matrices do not alter.

Another example are Quantum Dots, which is a nanocrystal technology of numerous cadmium atoms mixed with selenium coated with zinc sulfide which become excited and improve optical properties under UV light [8].

Some other strategies use colloidal gold nanoparticles that have been implemented with silver enhancement technology or combined with horseradish peroxidase to improve detection sensitivity and lower volume limits. For concurrent detection, combining colloidal gold nanoparticles and oligonucleotides of antibodies and antigens and two conjugate pads have been introduced to better practicability and abundancy [4].

Furthermore, alternative materials must also be taken into consideration in order to improve the function of the assay. An enhanced matrix must include three dimensions with alternating adequate pore size, highly regular surface, good fluid flow with good stability as well as multifunction characteristics in terms of application or reaction area.

Currently, it is appropriate to mention that there cannot be a single definition of a new and advanced assay. There are trends and concepts for new developments that must be recognized and approved or adjusted accordingly.

CONCLUSION

Undoubtedly, POCs, including lateral flow assays, present multiple benefits over traditional methods of testing. Its significant properties have enabled it to become a primary tool in testing for markers of diseases in medicine as well as food and environmental safety.

However, some essential features of LFAs must be altered and enhanced to meet the demands of the future diagnostic market, particularly including sensitivity, automation and integration of novel technologies.

LFAs must be more feasible and reliable in order to correspond with results from central laboratory systems.

Automation of its production as well as read out and processing of data must be improved. New materials and labelling techniques must be introduced to increase sensitivity and low volume analyte testing.

In combination with the advancements of the previous years regarding reproducibility and feasibility, LFAs are a promising diagnostic tool that can be used within or outside clinical settings contributing to enabling reliable diagnostic testing whenever and wherever it is needed.

SOURCES

1. INNOVA BIOSCIENCES Ltd.: INNOVA BIOSCIENCES GUIDE, Lateral flow immunoassays: Components, process, assay formats and nanoparticles. Cambridge. p. 3. https://fnkprddata.blob.core.windows.net/domestic/download/pdf/IBS_A_guide_to_lateral_flow_immunoassays.pdf (15.3.2022)

2. JAUSET-RUBIO, M., SVOBODOVÁ, M., MAIRAL, T. et al.: Ultrasensitive, rapid and inexpensive detection of DNA using paper based lateral flow assay. *Sci Rep*, 6, 2016, No. article 37732, p. 1-10.

3. KLONOFF, D.C.: Point-of-Care Blood Glucose Meter Accuracy in the Hospital Setting. *Diabetes Spectr*, 27, 2014, No. 3, p. 174-179.

4. KOCZULA, K.M., GALLOTTA, A.: Lateral flow assays. *Essays Biochem*, 60, 2016, No. 1, p. 111-120.

5. LEOWATTANA WATTANA, CHARURUKS NAVAPAN.: Critical Care and Rapid Response Testing in the Siriraj University Hospital in Thailand, Point of Care. *The Journal of Near-Patient Testing & Technology*, 4, 2003, No. 2, p. 253-257.

6. LIU, Y., ZHAN, L., QIN, Z. et al.: Ultrasensitive and Highly Specific Lateral Flow Assays for Point-of-Care Diagnosis. *ACS Nano*, 15, 2021, No. 3, p. 3593-3611.

7. LUPPA, P.B., MÜLLER, C., SCHLICHTIGER, A. et al.: Point-of-care testing (POCT): Current techniques and future perspectives. *Trends Analyt Chem*, 30, 2011, No. 6, p. 887-898.

8. MATHEW, T.K., TADI, P.: Blood Glucose Monitoring. *StatPearls*, August 2021 https://www.ncbi.nlm.nih.gov/books/NBK555976/ (21.2.2022)

9. O'FARRELL, B.: Evolution in Lateral Flow–Based Immunoassay Systems. *Lateral Flow Immunoassay*, 2008, p. 1-33.

10. RAJENDRAN, R., RAYMAN, G.: Point-of-care blood glucose testing for diabetes care in hospitalized patients: an evidence-based review. *J Diabetes Sci Technol*, 8, 2014, No. 6, p. 1081-1090.