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Lateral Flow Assay: A Point of Care Solution

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Abstract: ***Lateral flow assays (LFA) are the technology behind economical, easy, quick and transportable detection devices popular in biomedicine, agriculture, food and environmental sciences. This review presents an overview of the principle of the method and the critical components of the assay, focusing on lateral flow immunoassays. This type of assay has recently attracted considerable interest because of its potential to provide instantaneous diagnosis directly to patients, especially during pandemic. The range and interpretation of results and parameters used for the evaluation of the assay will also be discussed. The main advantages and disadvantages of LFAs will be summarized and relevant future improvements to testing devices and strategies will be proposed. Finally, the major recent advances and future diagnostic applications in the LFA field will be explored.***

Keywords: Nitrocellulose membrane, point - of - care, Rapid detection kit, capillary flow assay, Immuno chromatography assay, Nanotechnology, Multiplexing

1. Introduction

Lateral flow tests are frequently used for the rapid delivery of qualitative diagnostic results. The detection sensitivity of these immunochromatography assays is largely used for Point – of – care testing. This method, also known as the Lateral Flow assay, brings a simple and quick way to test a liquid sample of a patient, such as blood, urine, serum, saliva, sweat or other fluids, for a specific analyte [1]. The simplicity lies in the disposable test - strip design which incorporates all functions of an ELISA without the labour equipment. These tests are very versatile and used for point - of - care testing, at home, or in a laboratory with results typically within 30 min [2]. While the home pregnancy test is one of the traditional examples for this assay, existing use for infectious diseases and the incorporation of smartphone technology, indicate the massive potential of this technology—especially for underdeveloped nations.

Lateral flow assay (LFA) is another variation of the ELISA in which antigens are immobilized on a strip of cellulose or other suitable matrix. The sample is applied at the end of the strip and any antibodies present diffuse along it until they reach the position where the appropriate antigen is immobilized. Antibodies specific to the antigen bind to and remain at the site of the antigen and are visualized using labelled detection antibodies. The lateral flow test is not quantitative mostly, but is rapid and simple to perform, making it a suitable point - of - care test.

LFA - based tests are extensively used in hospitals, physician's offices and clinical laboratories for the qualitative and quantitative detection of specific antigens [3] and antibodies [4], as well as products of gene amplification [5, 6]. Different types of biological samples can be tested using LFAs, including urine [7], saliva samples [8], sweat [9, 10], serum samples [11], plasma [12], whole blood [13, 14] and other body fluids. Further in industries which LFA - based tests are employed include veterinary medicine [15], quality control procedures [16], product safety in food production [17], and environmental health and safety maintenance [18]. In these areas of utilization, rapid tests are used to screen for animal diseases [19], harmful pathogens [19, 20], dangerous chemicals [21], toxins [22] and water pollution [23, 24], among others.

Currently, there has been an increasing demand for point - of - care multiple diagnostic assays with multiple test lines allowing the rapid and real - time detection of multiple analytes contained in the sample. Such assays are easy to perform without the use of laboratory investigation, or individuals trained in chemical analysis. LFAs are quick as they are cost – effective to produce, easy to use and, importantly, widely accepted by users and regulatory authorities. This LFA technology is a favourable alternative for the advanced development of new pathways to invent novel technologies for the clinical diagnostic industries, usually, they require hundreds and millions of dollars and decades of work, but this process has the potential to produce devices that may become powerful tools for new challenging applications such as early cancer detection. Additionally, the long shelf life and the fact that freezing is not required for their storage, LFA is very well adapted for use in developing countries, small ambulatory care settings, remote regions and battlefields.

2. Principle of Lateral flow immunoassay:

The principle behind the LFAa liquid sample or its extract containing the analyte of interest moves without the assistance of external forces which is the capillary action through many regions of polymeric strip sticks, on which molecules that can interact with the analyte are attached. A typical lateral flow test strip consists of overlapping membranes that are mounted on a base card for better stability and handling. The sample is added at one end of the strip, on the adsorbent or the sample pad region, which is impregnated with buffer salts and surfactants that make the sample suitable for interaction with the detection system. The sample pad ensures that the analyte present in the sample will be capable of binding to the capture reagents of conjugates and on the membrane surface. The treated sample migrates through the conjugate release pad region, that is the conjugate pad, which contains antibodies that are specific to the target analyte and are conjugated to coloured or fluorescent elements–most frequently colloidal gold and latex microspheres. The sample, together with the conjugated antibody bound to the target analyte, moves along the strip into the detection zone. This has an absorbent membrane surface which is usually composed of nitrocellulose membrane with specific biological components mostly generally antibodies or antigens are used

immobilized in lines. Their role is to react with the analyte bound to the conjugated antibody. Recognition of the sample analyte results in an appropriate response on the test line, while a response on the control line specifies the appropriate liquid flow through the strip. The read - out, represented by the lines appearing with different intensities, can be assessed by eye or using a dedicated reader. To test multiple analytes simultaneously under the same conditions, additional test lines of antibodies specific to different analytes can be immobilized in an array format [26, 27]. On the other hand, multiple test lines loaded with the same antibody can be used for semi - quantitative assays. The principle of this assay is based on the step - by - step capture of colourimetric conjugate–antigen complexes by the immobilized antibody on each succeeding line, where the number of lines appearing on the strip is directly proportional to the concentration of the analyte particle [28, 29]. The liquid flows across the device because of the capillary force of the strip material and, to maintain this movement, an absorbent is attached at the end of the strip. The role of the absorbent pad is to wick the excess reagents and prevent the backflow of the liquid.

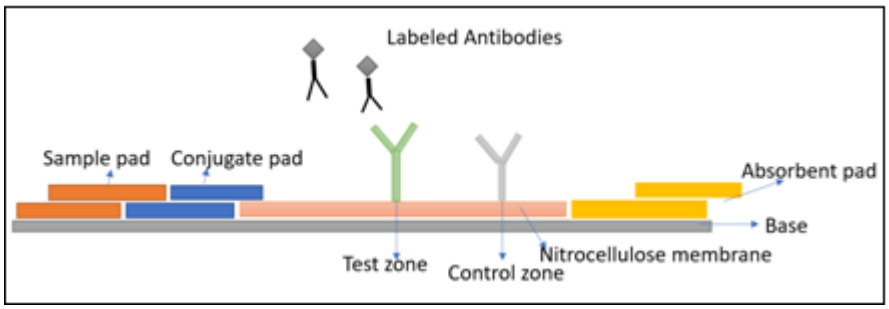

Figure 1: Lateral flow assay strip and components involved

Lateral flow assays can be developed to be used in a dipstick format or in a housed cassette. Both dipsticks and housed cassette assays will work in a parallel way, it is just dependent on the industry, sample matrix, and market necessity, as to which format is suitable.

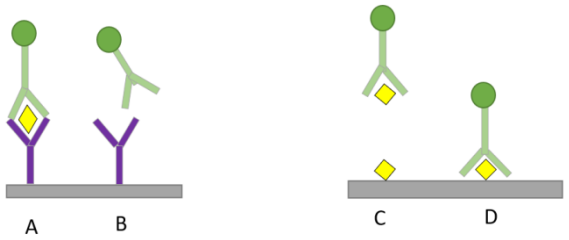

Figure 2: (A) The analyte is present so the binding occurs and the signal is seen. (B) No analyte hence no binding to read the signals. (C) Target analyte is present but no signal occurs due to the no binding. (D) No analyte but signal occurs due to proper binding

3. History

LFTs derive from the paperchromatography method, which was developed in 1943 by Martin and Synge, and elaborated in 1944 by Consden, Gordon and Martin. There was an explosion of activity in this field after 1945. [29] Later in 1971 the ELISA technology was developed.

4. Types of Lateral Flow assay

4.1 Sandwich Lateral flow assays

The sandwich assay format is typically used for detecting relatively large analytes. If the analyte has a minimumof two distinct binding sites (epitopes), a “sandwich assay” can be developed where an antibody to one epitope is conjugated to the nanoparticle and an antibody to another epitope is immobilized at the test line of the strip. The sandwich format results in a signal intensity that is comparable to the amount of analyte present in the sample.

4.2 Competitive Lateral flow assays

A competitive assay format is used for detecting analytes in which the analyte is very small for two antibodies to bind simultaneously, such as vitamins and antibiotics. In a competitive assay, the test line contains the target analyte molecule which is usually a protein - analyte complex. The nanoparticles are conjugated to an antibody that recognizes the analyte particles. If in case, the analyte is not present in the sample, the nanoparticle antibody conjugates will bind to the analyte at the test line, resulting in high signal intensity. If the target analyte is present in the sample provided, the analyte will bind to the antibodies on the nanoparticle and prevent the nanoparticle from binding to the test line region. This will reduce the signal at the test line resulting in a signal concentration that is inversely proportional to the amount of analyte present in the sample.

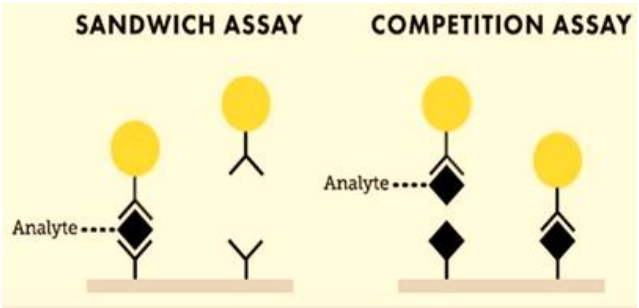

Figure 3: Sandwich lateral flow assay and Competition lateral flow assay

5. Components of the assay

The most common complications in the manufacture of LFA devices are caused by the hidden complication of the device. As the test is composed of many elements, problems can be

caused by material unsuitability, flaws in the connection of the overlapping elements or imperfect material characteristics. During the development of LFIAs (lateral flow immunochromatographic assay), most attention has been focused on finding the most suitable detection method or choosing the best antigen or antibody. However, it is very important to pay attention to all elements of the test, including the basic components such as the backing card, adhesive strip and cover tape, in order to produce a consistent and high - quality product.

5.1 Antibody

Although the physical components of the test strip, construction techniques and buffers play a major role in optimizing the test, the heart of these processes is the antibodies, which need to be carefully designed and highly purified. It is very important to ensure a consistent antibody supply with proven affinity and specificity. The use of monoclonal antibodies mostly derived from mouse hybridomas is preferable, as it allows the production of specific antibodies in large quantities.

5.2 Label

The lateral flow assays utilise conjugated gold, carbon, or coloured latex nanoparticles within the conjugate pad. Other labels include magnetic beads or coloured polystyrene beads. Regardless of the label types, they all perform the same purpose to create a three - way bond with antibodies and targets in order to make visible the control and test lines.

Labels will be chosen during lateral flow development depending on several factors such as the target, sample matrix and antibody. The optimisation of the assay will ensure the label interacts correctly with the antibody and antigen to ensure the efficiency and accuracy of results. This is vital for achieving a successful transfer and scale - up into routine lateral flow manufacturing.

The most important requirements of the nanoparticle label include:

- Colloidal stability in solution under various conditions and temperatures
- Susceptibility for detection over a large and useful dynamic range
- Efficiency and reproducibility of conjugation without the loss of chemical and biological integrity and activity
- Lack of or very low non - specific binding characteristics ensuring a high signal - to - noise ratio
- Commercial availability at a low cost
- Easy and scalable conjugation procedure.

Nowadays colloidal gold is the most widely used label in commercial LFIA. Although it can be prepared in the laboratory at low cost, there are many commercial sources available. It has an intense colour and no development process is needed for visualization. Moreover, it has high stability in both liquid and dried forms. Another popular label is latex, which can be tagged with a variety of detector reagents such as coloured or fluorescent dyes, and magnetic or paramagnetic components. As latex can be produced in multiple colours, it has an application in multiplex assays, which require discrimination between numerous lines. Carbon and fluorescent labels, or enzymatic modification [38] of the labels, are also used to improve the sensitivity of the assay. Carbon nanotubes have been shown to exhibit a limit of detection that is 10 - fold lower than that of gold. Fluorescent nanoparticles such as quantum dots may result in a high background noise which has been shown to be overcome by polymer encapsulation and surface blocking.

5.3 Membrane

The membrane is considered the most critical element in LFA strips and nitrocellulose is by far the most commonly used material. Moreover, there are also ‘pillar - based’ capillary LFA devices used for deoxyribonucleic acid (DNA) hybridization detection (where micropillar arrays replace the membrane), which have the advantage of more precise control of the capillary flow [33]. Important parameters characterizing a good membrane material are the capillary forces, as well as the ease of binding and immobilizing proteins necessary for subsequent selection, reaction and detection. A range of nitrocellulose pore sizes are available, from 0.05 to 12 μm. However, as the pores are not equally distributed because of the manufacturing process, capillary flow time is a more accurate parameter and it should be used when selecting the most effective strip material. The capillary flow time is the time required for the liquid to travel to and completely fill the strip of the membrane.

5.4 Sample pad

The sample pad can have multiple roles, the most important of which is to evenly distribute the sample and direct it to the conjugate pad. The sample pad is usually impregnated with buffer salts, proteins, surfactants and other liquids to control the flow rate of the sample and to make it suitable for interaction with the detection system. Moreover, the pores of the sample pad can act as a filter in order to remove redundant materials, e. g. red blood cells

5.5 Conjugate pad

The main role of the conjugate pad is to hold the detector particles and keep them functionally stable until the test is performed. This is ensured by the composition of the conjugate buffer, containing carbohydrates (such as sucrose), which serve as a preservative and a solubilization agent. When the conjugate particles are dried in the presence of sugar, the sugar molecules form a layer around them stabilizing their biological structures [39]. When the sample enters the conjugate pad, the sugar molecules rapidly dissolve carrying the particles into the fluid stream. It is crucial that the release is consistent between individual test strips.

5.6 Absorbent pad

The role of the absorbent pad is to wick the fluid through the membrane and to collect the processed liquid. The absorbent pad allows the use of larger sample volumes, which results in increased test sensitivity. The most popular absorbent pads are made of cellulose filters.

6. Detection method

Since the LFIA is an antibody - based technique, specificity and sensitivity may be affected by other chemicals with similar structures, leading to false positive results. The sensitivity of assays is limited by the K_d (dissociation constant) of the antibody–antigen conjugate and by the colorimetric read - out. In order to overcome these limitations, both readers and novel biochemical techniques have been developed to improve product quality and customer convenience. The selection of a detection system is mainly determined by the label employed in the analysis. Fluorescent dyes or paramagnetic particles cannot be detected directly by the naked eye and require dedicated readers for quantitative analysis. Moreover, automated detection methods provide advantages over manual imaging and processing in terms of time consumption, interpretation of results and adjustment of variables.

7. Pros and Cons

Many LFIAs are designed for use at point - of - care, providing low - cost, rapid and easy tests desirable in many industries. However, regulatory bodies often require confirmation of results using an independent method. Therefore, LFIA is only suitable for primary screening at point - of - care. Because of their long shelf life and the fact that refrigeration is not required for storage, these tests are very well adapted for use in developing countries. As the visual result is usually clear and easily distinguished, no additional specific equipment is needed. Research is ongoing to address some of the key weaknesses of LFAs, especially with respect to quantitative results. Data can be digitized using scanners or cameras with dedicated software, which will also allow the documentation of results. However, technological improvements will affect the cost of the apparatus and the duration of the analysis.

8. Current Global updates

The market expansion is attributed to the high incidence of infectious conditions coupled with the growing geriatric population and the increasing trend of point - of - care testing. The market is anticipated to also benefit from the increasing popularity of self - diagnosis, a prominent trend that is propelling the demand for home - based lateral flow assay devices. Amid the COVID - 19 crisis, the global market for Lateral Flow Assays estimated at US$7.8 Billion in the year 2020, is projected to reach a revised size of US$11.2 Billion by 2026, growing at a CAGR (Compound annual growth rate) of 5.9% over the analysis period. In addition, the integration of advanced quantification systems, new labels and simultaneous detection techniques is boosting growth. The COVID - 19 pandemic has presented an ideal growth opportunity for lateral flow assays due to the robust demand for lateral flow assay - based screening tests. This segment currently accounts for a 17.9% share of the global Lateral Flow Assays market. In contrast to laboratory - based tests that are time - intensive, lateral flow test kits are portable, convenient to use, and require minimal training.

The U. S. Market is Estimated at $3.3 Billion in 2021, While China is Forecast to Reach $872.4 Million by 2026

The Lateral Flow Assays market in the U. S. is estimated at US$3.3 Billion in the year 2021. The country currently accounts for a 38.64% share of the global market. China, the world`s second - largest economy, is forecast to reach an estimated market size of US$872.4 Million in the year 2026 trailing a CAGR of 8.8% through the analysis period.

Among the other noteworthy geographic markets are Japan and Canada, each forecast to grow at 3.8% and 5.5% respectively over the analysis period. Within Europe, Germany is forecast to grow at approximately 4.8% CAGR while the Rest of the European market (as defined in the study) will reach US$917 Million by the end of the analysis period.

The US market is at the forefront of the adoption of lateral flow assays supported by the rising prevalence of infectious conditions like tuberculosis and Lyme disease along with the increasing mortality rate linked with AIDS. Growth is further bolstered by the mayhem created by the COVID - 19 pandemic, prompting governments to make aggressive attempts toward mass screening programs. A host of factors are contributing to the rapid uptake of lateral flow assays in developing regions, especially Asia - Pacific.

While demand for these products is primarily being driven by growing healthcare expenditure and increased demand for diagnostics, other factors including rising healthcare awareness and subsequent increase in demand for healthcare services are also contributing to demand increase.

Nowadays, smartphones are indispensable personal devices. Smartphones have been used as infectious disease monitoring tools by identifying individuals who have been in contact with a patient. Finally, AI can also be used for analyzing everyday test results, which is something that has been demonstrated to be a sufficient strategy for disease surveillance by accurately identifying infected individuals. In addition to lateral flow assays, many other diagnostic methods have been incorporated with smartphones and AI for POC testing, such as RT - PCR, CRISPR/Cas, chest computed tomography and paper microfluidic devices [41, 42, 43, 44, 45, 46, 47]. These developments have revolutionized disease diagnosis by offering an approach to POC testing that is fast, accurate, cheap, and easy to use.

9. Discussion

The unique and remarkable properties of LFAs have contributed to the detection of disease biomarkers and infectious agents in medicine, agriculture, food and environmental safety. Although the principle of the method has remained unchanged for decades, there have been continuous improvements of LFA techniques leading to increased sensitivity and reproducibility, and the simultaneous detection of several analytes. Importantly, these assays can now be effectively performed outside the laboratory, providing great advantages for use in developing countries and at the point - of - care, whether in the field or in more traditional clinical settings.

10. Conclusion

Lateral flow assays (LFAs) can be used for the detection of proteins, nucleic acids and amplicons. LFAs are well established as a valuable tool in medical, veterinary, food, agricultural and environmental settings and for use in industrial diagnostics. The principle of an LFA is based on the movement of a liquid sample through a polymeric strip with attached molecules that interact with the analyte, providing a signal that can be visually detected. Although the concept behind the LFA is simple, the device has a complex architecture and many critical elements need to be considered during instrumental design. The most critical elements of the assay are the antibodies and the membrane, but attention should be paid to all of the materials used to ensure the compatibility and consistency of the product. An LFA is a fast, low - cost, portable and easy - to - use assay; however, the results are mostly qualitative (on/off) or semi - quantitative. An LFA is usually used for initial screening, which can be confirmed later by a fully quantitative method. LFA devices can be evaluated using parameters such as sensitivity, specificity and efficiency. Recent advances and future goals for improving LFAs are focused on identifying new signal amplification strategies, nanoparticle labels and quantification systems, as well as improving simultaneous detection.

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