Latest Improvement of Lateral Flow Assay in Detecting Nucleic Acid and Food Contaminant

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Abstract. Lateral flow assay is a paper chromatography used in portable test kits. It can show the testing results visually on the test line and control line. Lateral flow assay has been widely used during the COVID-19 pandemic due to its low cost and portability. Lateral flow assay was also used in the pregnancy test kits which displayed relatively high sensitivity. Lateral flow assay has an imperatively simple mechanism which is very convenient. This portable and inexpensive testing method has significant potential in ultrasensitive biosensing. Recently, numerous innovations in this technology are invented. With the combination of other new technology, the lateral flow assay could be modified. It could be improved in sensitivity and selectivity. In this work, the mechanism, latest research results, and applications were be discussed. The modification of LFA technology by CRISPR, fluorescence, and some nanoparticles were also introduced. This work would promote the further progression of lateral flow assay (LFA) and the possible usage of it.

Keywords: Lateral flow assay, applications, mechanism.

1. Introduction

Lateral flow assay (LFA) is a biosensing method which could be used to determine the appearance of specific molecules and show the results visually. LFA uses the capillary force to drive the liquid sample to flow through the nitrated cellulose membrane. In the testing process, the sample would react with different substances on the reaction zone, control line, and test line, eventually showing the results of testing.

Due to the low cost and ease of operation of this biosensing technique, this simple mechanism enables a lot of potential innovations. Recent innovations include the usage of pre-amplification, CRISPR, fluorescence, and other nanoparticles. Pre-amplification can amplify the signal and increase the sensitivity. When LFA was combination with clustered regularly interspaced short palindromic repeats (CRISPRs), the specific gene sequence could be located. Utilization of fluorescence and other possible nanoparticle can reinforce the accuracy.

Due to its high convenience, lateral flow immunoassay is widely used during the COVID-19 pandemic to detect the virus. The antigen LFA test has a relatively reliable mechanism and COVID-19 antigen test kits have been produced in a huge amount over the world. The UK has produced over 300 million test kits [1]. Thus, it is necessary to conclude the current study on the LFA technology and review the existing innovations. However, LFA still has the drawback of insufficient sensitivity, leading to a considerable amount of false-positive COVID-19 antigen test results [2]. In this work, the mechanism of LFA, the latest improvements, and applications will be discussed.

2. Mechanism

Different lateral flow assays have similar mechanisms. Take lateral flow immunoassay as an example, the mechanism starts with dropping the sample containing antibodies or other proteins to the sample pad. The sample could be saliva, blood, or urine [3]. The sample pad contains buffer salts, and other proteins to make the antibodies stable and ready to react with the detection reagents in the conjugate zone. As depicted in Fig. 1, the analyte flows to the conjugate zone containing conjugate agents and labels to bind with the antibodies, due to the capillary force. The conjugate pad needs to contain indicators that enable the test lines to be colored. The analyte of the antibodies and the
conjugate flow through the membrane to the test lines. The nitrocellulose membrane is responsible for the precise capillary flow [4]. The change of color on the test line should indicate the presence of targeted antibodies by recognizing the flow of the targeted analyte. The control line indicates whether the test is valid by recognizing the appearance of the analyte. Lastly, the analyte flows to the absorbent pad which absorbs the excess analyte.

![Schematic diagram of reaction process](image)

**Figure 1.** Schematic diagram of reaction process

3. Improvements

The widely used antigen LFA has the potential to strengthen the accurateness and selectivity. This could be done via the amalgamation of other bio-sensing technologies including DNA cleavage CRISPR, fluorescence, and other nanoparticles.

Most of the improved LFAs in detecting DNA segments make use of high affinity between the biotin and streptavidin and hybridization reaction. Streptavidin (SA), a tetrameric protein, has a high binding affinity with biotin [5]. Two complementary DNA single strands can undergo hybridization to form a double-strand DNA. With the aid of the SA and biotin and hybridization, it is possible to detect the DNA probe and successfully incorporate other technology. The following CRISPR, fluorescence, and nanoparticle-based LFA are all based on this SA biotin LFA.

The components of the improved LFA is composed of a sample of biotinylated DNA segments, SA-AuNPs complex, complementary single-strand DNA, and biotinylated antibodies. The AuNPs containing colloidal gold can show a red color [6]. The sample of biotinylated DNA segments is dropped into the sample port. The capillary force drives the sample through the nitrocellulose membrane and leads the sample to the reaction zone first [7]. The biotinylated DNA segments can bind to the SA-AuNPs complex because of the easy binding reaction between SA and biotin. The biotinylated DNA-SA-AuNPs complex will move forward as a whole. At the test line, a complex with a targeted DNA segment can bind with the complementary single-strand DNA fixed on the surface via hybridization. The untargeted ones are unable to undergo such hybridization. The remaining complex moves to the control line where the complex containing SA binds with the biotinylated antibody. Thus, the test line result can indicate the presence of the targeted DNA, and the control line can indicate the validity of this test [8]. Most of the innovation can combine with this biotin SA mechanism to successfully integrate other mechanisms or particles into the LFA reaction.

In recent researches, many technologies or new materials have been combined with this technology to improve the performance. Among them, the modification of LFA technology, which was combine by CRISPR, fluorescence, and some nanoparticles, is particularly noteworthy. The following modification methods will be introduced separately.
3.1. CRISPR-based LFA

With the help of the CRISPR before the lateral flow biosensing step, the sensitivity and selectivity can be improved. As shown in Fig. 2, CRISPR is a protein-RNA complex that can locate and cut the targeted DNA sequence. The sgRNA (single guide RNA) leads the complex to locate and bind to a specific DNA sequence called PAM. When sgRNA binds with the PAM sequence, CRISPR will undergo cleavage activity [9]. Based on CRISPR, lateral flow assay can be used to sense the presence of specific DNA sequences. The CRISPR-based LFA needs pre-amplification of the sample to improve the sensitivity. The reaction used biotin and SA.

**Figure 2. CRISPR Cas9 Reaction**

Firstly, the CRISPR reaction is performed. With the presence of the targeted DNA segments, cleavage activity will take place. Thus, the sample containing targeted DNA segments will have two cleaved segments, and the sample without the targeted one will have one segment. After the CRISPR step, the biotin-SA LFA mentioned above will take place. At the test line, complexes with an uncleaved DNA segment can bind with the complementary single-strand DNA fixed on the surface. The cleaved ones are unable to undergo hybridization. The remaining complex moves to the control line where the complex containing SA binds with the biotinylated antibody [10]. Thus, if there’s a presence of the targeted DNA segments, only the control line will be red. If there’s an absence of the targeted DNA segments, both lines will be red. This LFA improves the selectivity; however, it depends on the pre-amplification to raise the signal [11].

3.2. Fluorescence

The fluorescence-based LFA can be used to detect the antigen and the DNA segment. The fluorescence-based LFA has the significant advantage of sensitivity and it is able to detect samples with low concentration of targeted molecule. For example, fluorescent carbon nanoparticles (FCN) have proved to be an effective innovation in increasing the sensitivity of LFA. FCN is attached to complementary DNA segments that are fixed on the surface of LFA and this segment can undergo hybridization with the targeted DNA segment. Thus, with the similar mechanism of leaving targeted DNA segment complex containing FCN on the control line. The result can be read by a fluorescent reader. This FCN-based LFA is able to detect very low-concentration DNA segments sample of 0.4 fM. [12] The usage of fluorescence largely increases the sensitivity but it has the drawback of reducing portability. Although there are relatively small and portable fluorescent readers. However, there should be doubt about whether the fluorescent enhanced LFA can be used widely as the COVID-19 antigen test kits.
3.3. Other Nanoparticles

There is an abundance of literature discussing the possible nanoparticles and following reactions that can be used to innovate the LFA. It was reported that glycosylated gold nanoparticles and iridium oxide (IV) nanoparticles are introduced to modify the LFA. Glycosylated gold nanoparticles have a high binding affinity to the toxin and iridium oxide (IV) nanoparticle with a blue color is considered an alternative dye to the colloidal gold [13]. For the glycosylated gold nanoparticles, the biotin SA mechanism is replaced by the binding between the glycosylated gold complex and other toxins. The glycosylated polymers are also easy to generate and there are available pathways to generate them [14]. To improve the visual reading of LFA, Iridium oxide (IV) is believed to be a potential replacement for the colloidal gold dye. Iridium oxide (IV) NPs complex shows a blue color. It also has assumable binding properties to the complex. It has rather stable properties in showing the color as an indicator compared to the AuNPs complex. For example, the colloidal gold nanoparticle is likely to aggregate in the salt-impregnated samples, while the iridium oxide (IV) nanoparticle is more stable in such samples. Other light-initiated nanoparticles are also available for the improvement in sensitivity [15].

4. Applications

4.1. Nucleic Acid

Lateral flow assay is largely used during the COVID-19 pandemic all over the world. Due to the portability and low cost, many countries have produced huge amounts of COVID-19 antigen test kits. In the United Kingdom, millions of kits have been purchased by the government. The rapid diagnostics of lateral flow assay make it suitable for testing in home and near-patient testing. However, the problem of low sensitivity is still reported. The sample of mucus from the nose capacity contains the antigen that can bind to the corresponding antibodies on different reaction zones. Thus, the presence of the targeted antigen or the positive will give out the result of two red lines and the negative result will be only the red control line. The LFA test kits are used for various diseases including HIV, influenza, etc. Other diseases like dengue or chlamydia can also be detected via other mechanisms besides nucleic acid detection.

4.2. Food Contaminant

Food contaminant LFA utilizes the similar mechanism of capturing conjugate molecules on the test line or control line. The results of such LFA are shown visually by activating the colloidal gold dye. The portable LFA test can be used in detecting food contaminants like mycotoxins and phycotoxins [16]. The conjugate carrier protein is prepared and fixed on the surface of the nitrocellulose paper, and it can retain the corresponding toxin. The fast speed of the testing and simple visual reading of the results creates huge potential for the development of LFA in the aspects of food contaminants detection.

5. Conclusion

Lateral flow assay can be the most widely used paper chromatography for the public. LFA shows great potential due to its simple mechanism, low cost, and high portability. The widely used pregnancy test kits and COVID-19 antigen test kits have shown the great success of the usage of lateral flow assay in medical aspects. The development of LFA is inspiring for many bio-sensing technologies. The testing results could be visually displayed with a simple mechanism. To incorporate with other technologies, the SA and biotin binding reaction are designed into the lateral flow mechanism. As it is discussed in this work, the incorporation of CRISPR, fluorescence, and nanoparticles into LFA has shown exciting progressive results. In addition, future studies can focus on a large amount of manufacture of these improved lateral flow assays. The future application of
this technology can also become more specific to the scale of DNA segments. The test kits for detecting food contaminants can also be refined. The development and improvement of the components of lateral flow assay are also promising. More application fields are worth exploring.

References