Applications of CRISPR-based Lateral Flow Assays in Medicine

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Abstract. Due to the constant development of medicine, CRISPR-based lateral flow assays (LFAs) have become a promising technique for detecting various diseases and pathogens. A lot of researches concerning with CRISPR-Cas have been done. Its performance would be enhanced when it was combined with LFAs. In this way, a simple, rapid, and cost-effective diagnostic test could be achieved. This work points out the applications of CRISPR-based LFAs in medicine, specifically in virus detection and bacterial detection. In terms of virus detection, this review focuses on detecting SARS-CoV-2, HBV, and EBV. In terms of bacterial detection, this review highlights the detection of Salmonella, Leptospira, and Staphylococcus aureus. These assays are all rapid, accurate, simple, time-saving, and cost-effective. Despite the advantages of CRISPR-based LFAs, there are also limitations and challenges that need to be addressed, which are mainly centered on the sensitivity and target range of current assays. To overcome those limitations, future directions should focus on areas such as multiplexing, integration with digital technologies, point-of-care testing in situations with limited resources, and the potential for CRISPR-based therapeutics.

Keywords: CRISPR-Cas, lateral flow assay, medicine, detection.

1. Introduction

Since medicine is closely related to everyone's life, the detection of diseases has become the focus of attention. An optimal diagnostic platform must be created in order to detect a variety of pathogens and diseases quickly, sensitively, specifically, and affordably [1]. It should be simple to use and capable of providing instrument-free readouts for early pathogen detection [1].

In this day and age, the development and applications of CRISPR-based technologies have completely changed the field of molecular biology. Among these advancements, a CRISPR-based LFA has become a promising tool for rapid and accurate diagnosis in medicine. These assays use the CRISPR-Cas system to detect particular DNA sequences in a sample that can be used to diagnose various diseases [2]. The LFA is an easy and quick diagnostic tool that can be used in situations with limited resources. The combination these two techniques would make medical diagnostics even more advanced.

CRISPR-Cas has been used widely in various areas, including medicine. The two components of the system are the guide RNA and the Cas protein. The Cas protein is an endonuclease that can cleave DNA at a particular place, and the guide RNA directs the Cas protein to the target region [3]. Through the use of the guide RNA to recognize particular DNA sequences in a sample, CRISPR-Cas has been modified to diagnose plenty of diseases.

The LFA is an easy and quick diagnostic tool that can be used in situations with limited resources. They can identify the existence of a target substance in a liquid sample for a short time without requiring very sophisticated equipment. At first, the sample pad receives the sample containing antibodies or other proteins. The detection reagents can react with the conjugate pad, which includes antibodies that have been joined to gold nanoparticles. Those antibodies bind to the target molecule, and on the nitrocellulose membrane, the gold nanoparticles can be visualized as a red line. The absorbent pad wicks the sample through the strip, and the result can be shown within a few minutes.

The combination of CRISPR-Cas and LFAs possesses the capacity to reinforce the field of medical diagnostics. CRISPR-Cas can be utilized to detect specific DNA sequences in a sample, while the LFA can provide a simple and rapid readout [4]. This technology has been applied to detect various diseases. Thus, this review will summarize some applications of CRISPR-based LFAs in medicine, including virus detection and bacterial detection.

2. Applications of CRISPR-based Lateral Flow Assays in Medicine

2.1. Virus Detection

CRISPR-based LFAs have developed into highly valuable techniques for virus detection. Notably, they have been successfully applied in detecting various viruses such as SARS-CoV-2, HBV, and EBV. These assays provide numerous advantages, including simplicity, sensitivity, and cost-effectiveness, making them effective tools for disease detection and diagnosis.

Osborn et al. introduce a fluorescence-based assay that can detect SARS-CoV-2 [5]. SARS-CoV-2 can be detected by using CRISPR-Cas based LFA [5]. The assay can be modified to be used in a lateral flow format, making it appropriate for usage in environments with minimal resources.

Huang et al. also propose a CRISPR-based LFA used to identify SARS-CoV-2. The assay has a high sensitivity and specificity for viral detection. Additionally, the advantages of traditional RNA-targeting methods are combined in the CRISPR-based LFA. With a setup time of less than 15 minutes, the data can be analyzed in an hour using either a lateral-flow readout or a fluorescence readout. This suggests that the preparation process is quicker than RT-qPCR assays, which demand a lot of equipment [6]. Therefore, without the need for sophisticated equipment, this assay can be utilized to quickly diagnose COVID-19.

A CRISPR-based LFA is also suitable for detecting HBV, which results in hepatitis B, an infectious disease. Ding et al. describe a quick and precise point-of-care assay for HBV detection. The assay results can be visualized using both lateral flow test strips and fluorescent readout, giving results within just 20 min [7]. These two methods have extremely promising sensitivity and specificity when compared to qPCR. Because this assay requires less equipment and offers inexpensive, quick, and accurate test results, it is incredibly useful for point-of-care HBV detection [7].

Furthermore, Nasopharyngeal Carcinoma (NPC), which is a prevalent malignant tumor of the globe, is brought on by the presence and growth of EBV. Yuan et al. propose a straightforward and sensitive method to detect EBV based on integrating CRISPR-Cas12a with a LFA [8]. This assay shows great specificity in the presence of different viruses and can be used with serum samples from patients with EBV Burkitt's lymphoma [8]. EBV and other non-infectious diseases can be detected with this technology in the future.

2.2. Bacterial Detection

CRISPR-based LFAs have also emerged as ideal methods for bacterial detection, including detecting salmonella, leptospirosis, and staphylococcus aureus. These assays highlight the potential of CRISPR-based diagnostics in revolutionizing bacterial detection, providing fast and precise results at the point of care.

Wang et al. summarize the progress of a CRISPR/Cas9-integrated lateral flow strip (Cas9-LFS) that can detect salmonella quickly and precisely. The potential of CRISPR/Cas-based biosensors has been characterized as a modern approach to harmful bacterial identification, which can cover the gaps in existing detection methods that are cumbersome and inconvenient [9]. To conclude, CRISPR-based diagnostics have evolved as a clinically viable diagnostic technique for its quick, simple, and ultrasensitive identification of biomarkers at the point of care.

In addition, CRISPR-Cas has been utilized to detect Leptospirosis, which occurs mainly in rural areas. Using CRISPR technology, Natarajan et al. present a LFA to identify the Leptospira lipL32 gene, which has a high sensitivity and specificity for detecting the target gene. The lateral flow test strip can be utilized for on-field testing because it is made portable [10]. DNA collected from clinical samples of patients as well as samples from animals linked to the reproductive condition can be employed in the devised test strip as a sensitive method to diagnose leptospirosis [10].

Zhou et al. introduce a fluorescence-enhanced LFB on the basis of CRISPR/Cas12a for detecting staphylococcus aureus, which is a common foodborne pathogenic bacterium. Under ideal circumstances, the functionalized quantum dots can be combined with recombinase-aided amplification (RAA). It was reported that a very low limit of detection, about 75aM, could be reached

[11]. Staphylococcus aureus may be swiftly and precisely found using this technology in samples of natural meat and vegetables, protecting consumers from food illness.

3. Conclusion

CRISPR-based LFAs have emerged as a powerful tool in medicine, offering rapid and accurate detection methods for various applications, including virus detection and bacterial detection. These assays combine the specificity of the CRISPR system with the simplicity of LFAs, providing a user-friendly and cost-effective diagnostic solution.

In contrast, there are also many limitations and challenges of CRISPR-based LFAs. First of all, the sensitivity of current CRISPR-based LFAs is not good enough. They have to be paired with an amplification process. Secondly, the target range of this method is limited. They are only specific to the particular target molecules they are designed for; however, they are not suitable for detecting a wider range of targets. Moreover, there is still a risk of off-target effects, which may contribute to false-positive or false-negative results.

Future directions for the advancement of CRISPR-based LFAs include multiplexing, integration with digital technologies, point-of-care testing in situations with limited resources, and the potential for CRISPR-based therapeutics. Multiplexing will allow for detecting multiple pathogens or biomarkers in a single sample, which will improve the effectiveness and precision of the assay. Integrating CRISPR-based LFAs with digital technologies, such as smartphones or portable readers, can enhance the sensitivity. This will also enable real-time monitoring and data analysis. The development of CRISPR-based LFAs for point-of-care testing in situations with limited resources is another area of focus, which will enable quick and precise diagnosis of diseases or pathogens in remote or backward areas. Furthermore, the potential for CRISPR-based therapeutics will allow for targeted and precise treatment of diseases at the molecular level.

To sum up, CRISPR-based LFAs have numerous advantages in medicine. The ongoing advancements in the CRISPR system and LFAs are expected to further enhance the abilities of CRISPR-based LFAs in the future.

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