CRISPR-based Microfluidic Biosensor for the Diagnosis of Infectious Diseases in Remote Areas of Peru

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Abstract

The following technological proposal is based on the design of a low-cost microfluidic diagnostic device that integrates the CRISPR/Cas system for the detection of the SARS-CoV-2 gene or other pathogens. In other words, this device would represent a versatile point-of-care technology, capable of having a positive impact on the diagnosis of infectious diseases; and consequently, it could help in the prevention of future epidemics in areas with low health service coverage in the country.

Introduction

COVID-19 is an infectious respiratory disease caused by the SARS-CoV2 virus. Its spread has caused a great loss of life and economic crisis worldwide. Diagnostic tools are currently available, such as, quantitative real-time PCR (qPCR), which is the most widely used diagnostic test for COVID-19, viral antigen detection assays and serological tests that indicate the presence of SARS-CoV-2 specific antibodies [1] (Fig. 1)

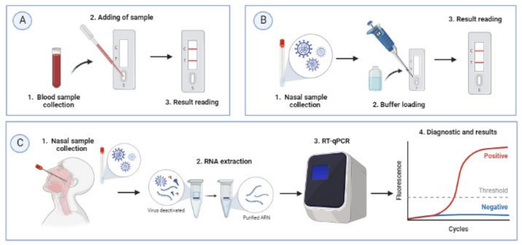

Figure 1. Conventional COVID-19 diagnostic techniques. A. Rapid serological test. B. Viral antigen test. C. Real-time PCR (RT-qPCR) Source: Own elaboration. Developed with BioRender.

However, the pandemic revealed limitations in these types of diagnostic tests, including limited availability of valid test kits and certified testing laboratories, complex testing workflows, and expensive laboratory instrumentation and materials for sample and reagent management [2].

In that regard, the high cost, prolonged diagnostic time of PCR testing, and low sensitivity of point-of-care rapid tests directly contributed to society's inability to efficiently identify COVID-19 positive individuals [2]. For this reason, the development of rapid, sensitive and low-cost diagnostic devices has become a clinical challenge of great interest in recent years. All this, with the aim of facilitating the appropriate isolation of infected individuals and the treatment of patients.

Objectives

General objective:

- Propose a design for a low-cost point-of-care diagnostic device for pathogen detection in remote areas of Peru.

Specific objectives:

- Integrate the CRISPR system with a microfluidic device for the development of a cost-effective and easy-to-use diagnostic platform.
- Develop a mobile application for analysis and interpretation of results.

Methodology

A. Detection Method

A CRISPR-Cas system is able to identify and bind to a specific DNA or ARN sequence using a guide ARN (gRNA) complementary to the target sequence. In particular, the Cas13 protein in its active form cleaves nearby ARN sequences. Therefore, fluorescence reporters can be used to indicate the presence of a viral ARN [3].

In addition, magnetic beads can be used for the extraction of RNA molecules from a blood sample. These beads bind to the RNA and are then attracted by a magnet. This procedure can be implemented in a microfluidic system according to [4].

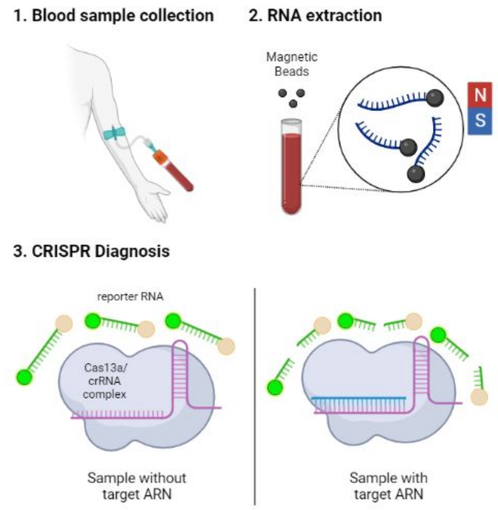

Figure 2. Methodology for the detection of viral ARN using a CRISPR-Cas13a complex and magnetic pellets for RNA extraction. Source: Own elaboration. Developed with BioRender.

These process will be implement in a modular microfluidic device. The advantages of microfluidics systems include rapid diagnosis with small quantities of patient sample, lower reagent consumption, high reproducibility, and easy implementation [5].

B. Analysis and interpretation of results.

The mobile application allows to evaluate the signal detected by the fluorescence-based reading method.

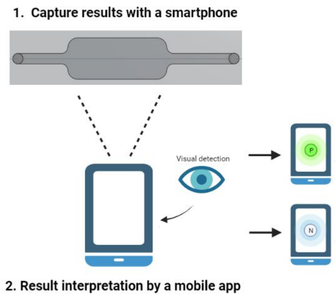

Figure 3. Mobile application for analysis and interpretation of results. Source: Own elaboration. Developed with BioRender.

Design

The microfluidic device is composed of 3 modules in series. The modules are: Module A: RNA washing, Module B: RNA elution and Module C: CRISPR sensor.

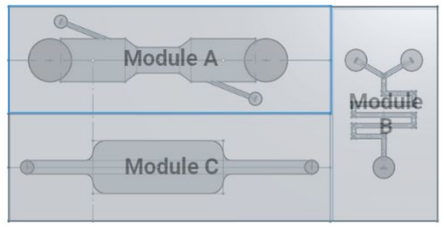

Figure 4. *Design of the device and separation of its modules. Designed in Onshape program.*

The design is based on the adaptation of the last steps purification of RNA in very small amounts. The CRISPR-based Microfluidic Biosensor can detect these small RNA concentrations and we obtain a measurable signal in the last module of the device.

A. RNA washing

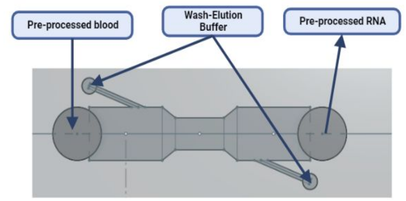

Figure 5. RNA washing module with its two inputs: preprocessed blood and buffer to get the output: unpurified RNA output.

B. RNA elution

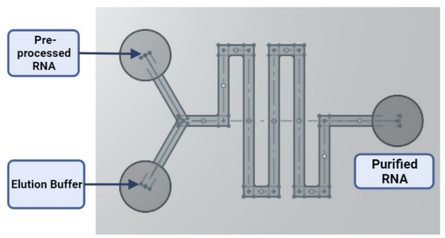

Figure 6. RNA elution module, where the complete purification of RNA takes place.

C. CRISPR SENSOR

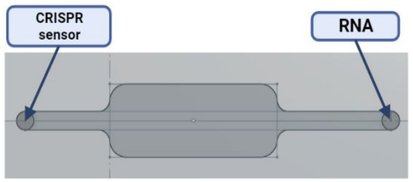

Figure 7. Module where RNA is combined to the CRISPR-Cas13a sensor. The RNA concentration obtained will be directly proportional to the fluorescence of the sample.

Estimated budget

The total cost of materials used for the fluorescence-based CRISPR-mediated microfluidic diagnostic test would be lower than the RT-qPCR test. According to [3], the initial instrumentation costs for CRISPR-based tests are significantly lower. In that sense, the cost of a single reaction for the CRISPR-COVID assay would be less than $3.5 on a research scale and would come down to $0.6 on a production scale [6].

Scope and Social Impact

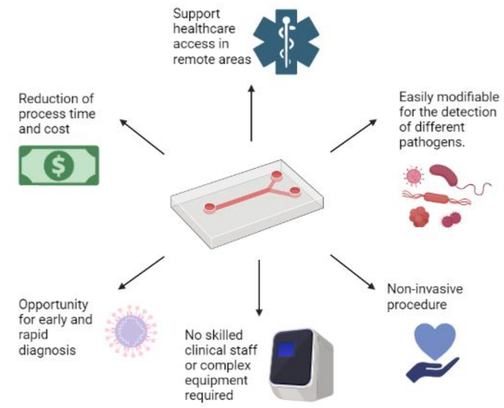

Figure 8. Advantages and scope of a point-of-care diagnostic device.

Conclusions and future perspectives

- A simple, low-cost microfluidic system was designed for the detection of SARS-CoV-2 and other pathogens and can be used without the need of a skilled clinical staff and complex medical equipment.
- This biosensor is versatile, because it can be implement for the detection of other pathogen RNA molecules by simple changing the crRNA of the CRISPR-Cas13a complex.
- As a future work, it is proposed to establish connections between the three modules in order to automate the diagnostic test and reduce user manipulation.
- The biosensor has the potential to be a viable alternative for the early diagnosis of common infectious diseases in remote areas of Peru, such as dengue, zika, among others.

References

[1] X. Wu, Q. Chen, J. Li, and Z. Liu, "Diagnostic techniques for COVID-19: A mini-review," J. Virol. Methods, vol. 301, p. 114437, Mar. 2022, doi: 10.1016/J.JVIROMET.2021.114437.

[2] N. Li et al., "Overcoming the limitations of COVID-19 diagnostics with nanostructures, nucleic acid engineering, and additive manufacturing," Curr. Opin. Solid State Mater. Sci., vol. 26, no. 1, p. 100966, Feb. 2022, doi: 10.1016/J.COSSMS.2021.100966.

[3] F. Palaz, A. K. Kalkan, A. Tozluyurt, and M. Ozsoz, "CRISPR-based tools: Alternative methods for the diagnosis of COVID-19," Clinical Biochemistry, vol. 89, pp. 1–13, Mar. 2021, doi: 10.1016/j.clinbiochem.2020.12.011.

[4] Z. Li, X. Ding, K. Yin, L. Avery, E. Ballesteros, and C. Liu, "Supporting Information Instrument-Free, CRISPR-based Diagnostics of SARS-CoV-2 using Self-Contained Microfluidic System".

[5] B. Nasseri, N. Soleimani, N. Rabiee, A. Kalbasi, M. Karimi, and M. R. Hamblin, "Point-of-care microfluidic devices for pathogen detection," Biosensors and Bioelectronics, vol. 117, pp. 112–128, Oct. 2018, doi: 10.1016/j.bios.2018.05.050.

[6] J.S. Gootenberg, et al., "Nucleic acid detection with CRISPR-Cas13a/C2c2" Science, 356 (6336) (2017), pp. 438-442, doi:10.1126/science.aam9321