Review 2: "An integrated lab-on-a-chip device for RNA extraction, amplification and CRISPR-Cas12a-assisted detection for COVID-19 screening in resource-limited settings"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

The article is structured and well organized. The significance of the study enumerates the need for a sensitive biosensing technique for SARS-CoV-2 detection. The uniqueness of this work lies in the integration of all steps in a single device and the compatibility of the method with saliva samples as an alternative to the irritating nasopharyngeal sampling. However, the main concepts and choice of approaches and procedures lack justification. In this aspect, whatever approaches the researchers adopted must be justified such as the use of saliva over nasal swab, and the use of Cas12a instead of Cas9 or Cas13. This issue raises the question of nobility and arises doubt over the choice of approach. Decision-makers need to evaluate this work carefully before publishing it. There is also a need for a video demonstrating the step-by-step procedure and results.

Summary: The authors proposed a reliable, affordable, rapid, and high-precision lab-on-chip system named IFAST-CRISPR for the detection of SARS-CoV-2. The study utilized saliva samples where the virus is extracted and amplified prior to detection using a readout device. The microfluidic device is fabricated with a CNC machine using polymethyl methacrylate polymer known as engineering plastic which has several characteristics such as being transparent, thermoplastic, and light. The overall method includes device design and fabrication followed by DETECTR assay using RNA extraction, RT-LAMP reaction, and CRISPR-Cas12 detection assay, and finally the combination of all steps for the detection of SARS-CoV-2. The authors' main conclusion is the microfluidic lab-on-chip CRISPR-Cas12a device known as IFAST-CRISPR achieved similar sensitivity and specificity with RT-qPCR and they claim that the study presents a simple, affordable, and versatile diagnostic device for COVID-19.

Major comments:

1. Even though RT-PCR is regarded as the gold standard for the detection of SARS-CoV-2, several underdeveloped countries cannot afford it. Thus, most of the hospitals rely on radiographic imaging based on lung ultrasound, chest X-ray, and CT scan which has proven to be efficient, cheap, and easy. This article needs to address how the proposed system can better than the diagnostic techniques such as radiography.
2. Several studies have shown that the use of the Cas13 enzyme is highly effective for the detection of SARS-CoV-2, even without a nucleic acid amplification step. Therefore, it must be also justified why the authors chose Cas12a.
3. Evaluation of biosensing techniques requires many procedures such as reproducibility and linearity, etc. However, the authors need to validate their platform using these types of tests which is fundamental.

Minor comments:

1. Authors need to proofread the work to adjust grammar errors and typos.
2. The authors can present a table that summarizes the main studies on CRISPR (Cas3, Cas9, Cas12, Cas13, etc.)-based SARS-CoV-2 detection studies and compare them with the study in hand.
3. A supplementary video demonstrating the step-by-step procedure and results can be presented.
4. The authors can enumerate the research contribution below the significance of the research.
5. The binding of SARS-CoV-2 RNAs to silica paramagnetic beads could be explained based on chemistry.