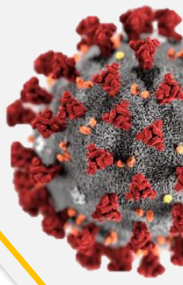
A SMARTPHONE-READ QUANTITATIVE SALIVA TEST FOR COVID-19



PURPOSE

To provide scientific evidence on the effectiveness, safety and cost-effectiveness of smartphone-read saliva tests for the detection of COVID-19.

INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused great impact to health and economy. Globally, as of 3:42pm CET, 10 January 2021, there have been 88,383,771 confirmed cases of COVID-19, including 1,919,126 deaths, reported to WHO.[9]

The molecular tests Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) technology is the recognised method by the World Health Organization (WHO), US Center for Disease Control (CDC) and Ministry of Health Malaysia (MoH) for confirmation of COVID-19 case but this method require a swab sample and trained staff with time consuming laboratory procedure.[4,9] As a result, there is a delay of hours to many days between when tests are taken, and results are obtained. Thus, to expand COVID-19 testing capacity, new assays should use samples that can be easily collected, have greater sensitivity than the current reference standard, allow viral quantification for treatment monitoring, require minimal training and equipment to obtain valid, robust and quantitative results.

A researcher group from Tulane University, New Orleans have developed a portable, Real Time-Recombinase Polymerase amplification (RT-RPA) CRISPR-Fluorescent Detection System (FDS) assay to sensitively quantify SARS-CoV-2 present in saliva, without RNA isolation, and adapted this assay to a chip format that was a smartphone reader. This saliva-based detect COVID-19 within 15 minute sample-to-answer time and does not require RNA isolation or laboratory equipment.[7]

Saliva samples offer practical and logistical advantages for diagnostic and screening efforts, since they can be directly collected by the patient, reducing the need for, and exposure risk of, medical personnel.[6,10,12]

This assay uses CRISPR/Cas12a activity to enhance signal from an amplified viral RNA target, which is stimulated by laser diode integrated into a smartphone based fluorescence microscope readout device. This test uses the same CRISPR-based approach that the researchers have submitted to the Food and Drug Administration for Emergency Use Authorization.[3,6]

This device incorporated a smartphone socket, external lens filter, laser diode powered by AAA batteries, a power switch, chip slot, and emission filter for the smartphone camera. The field of view (FOV) of this device was increased relative to previous smartphone microscope designs by adding an external lens with a 50 mm focal length. This yielded a FOV compatible with diameter of the reaction well array on assay chip without producing significant aberration. This device also employed a 100 mW laser diode with a high incidence angle to allow sensitive detection of reaction products while minimizing background noise.[5]

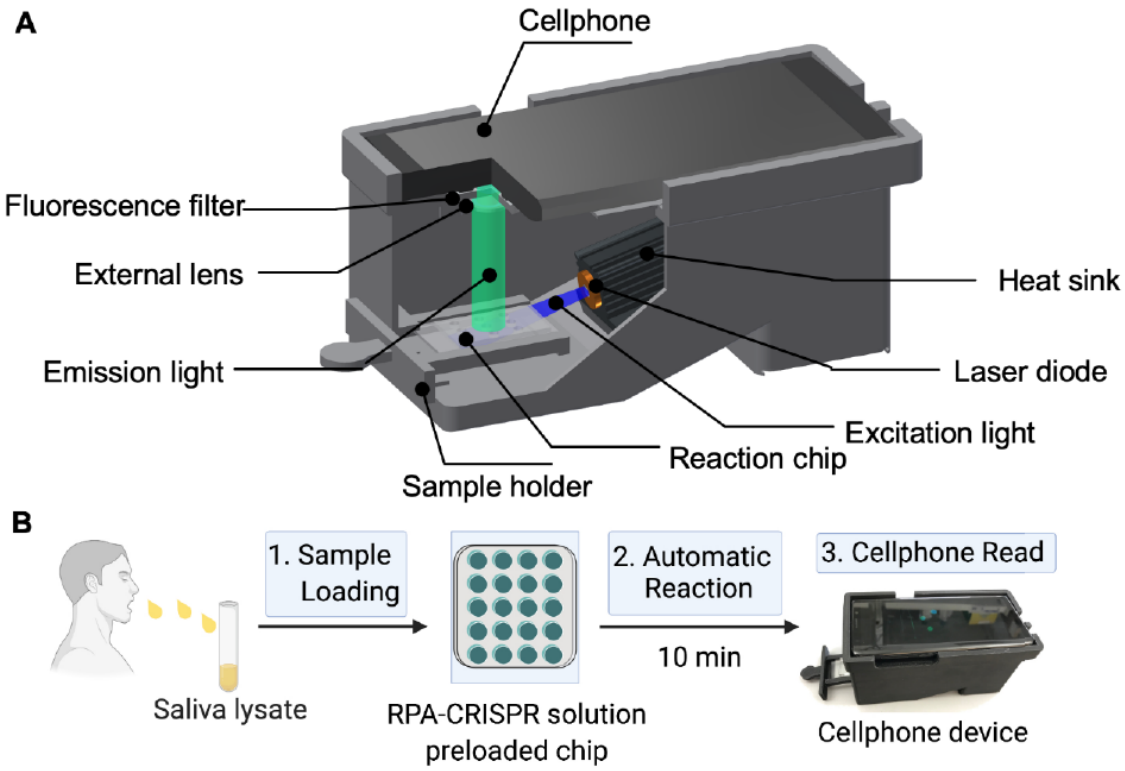


Figure 1: Design of a smartphone-based assay reader and its analytical and diagnostic performance when employed to read on-chip CRISPR assays. (A) Schematic of a 3D-printed smartphone fluorescence reader. (B) Workflow of a saliva-based on-chip CRISPR-FDS smartphone assay.[5]

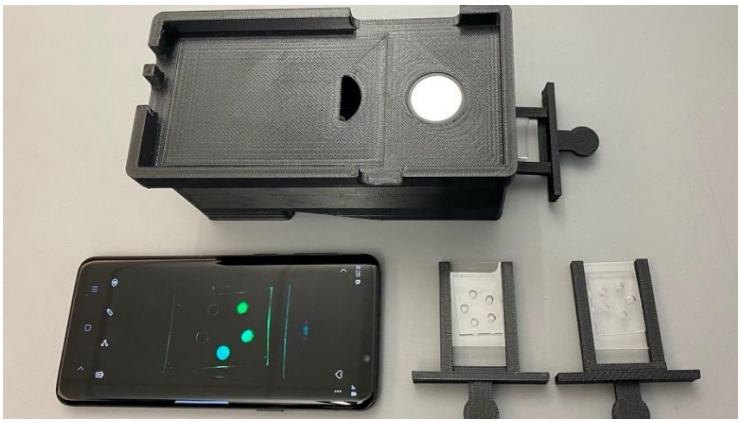


Figure 2: The prototype device of a smartphone-based assay reader.[5]

EVIDENCE/INFORMATION SUMMARY

Systematic search through Ovid interface, FDA website and Google Scholar search engine retrieved one scientific evidence on saliva based on-chip CRISP-FDS smartphone assay and 133 scientific evidence on detection of COVID-19 by using saliva specimen.

Recent research from the Yale School of Public Health showed that saliva specimen (mean log copies per milliliter, 5.58; 95% confidence interval [CI]; 5.09, 6.07) contains more SARS-CoV-2 RNA copies than in nasopharyngeal swab specimens (mean log copies per milliliter, 4.93; 95% CI; 4.53, 5.33). A total of 70 inpatients with Covid-19 participated in the study to evaluate the sensitivity of saliva specimen as compared to the nasopharyngeal swab specimen. A higher percentage of saliva samples than nasopharyngeal swab samples were positive up to 10 days after the Covid-19 diagnosis. At one to five days after diagnosis, 81% (95% CI; 71, 96) of the saliva samples were positive, as compared with 71% (95% CI; 67, 94) of the nasopharyngeal swab specimens.[12] These findings suggest that saliva specimens and nasopharyngeal swab specimens have at least similar sensitivity in the detection of SARS-CoV-2 during the course of hospitalisation.

Results from the analysis done by the researchers from Tulane University demonstrated good linearity (R^2 = 0.90) across a broad range of virus concentrations (1-10^7 copies/µl), with a LOD of 0.05 copies/µL. Subsequent CRISPR-FDS analysis of 20 replicate samples containing 0.05, 0.1 and 0.25 copies/µL detected positive signal in all samples, suggesting the actual LOD is <0.05 copies/µL. CRISPR-FDS demonstrated complete concordance (100% positive and negative percent agreement) with RT-qPCR when analysing saliva aliquots spiked with or without virus, at concentrations matching one times to two times the LOD (0.05 and 0.1 copies/µL, respectively) of the CRISP-FDS assay.[5]

These studies were followed by CRISPR-FDS evaluation of the time course of SARS-CoV-2 RNA expression in animal using nasal and pharyngeal swab samples of a non-human primate of COVID-19. Paired nasal and oropharyngeal (saliva analog) swabs were obtained from seven non-human primates before and after SARS-CoV-2 infection. CRISPR-FDS determined that mean SARS-CoV-2 RNA levels were 3.6-fold to 124-fold higher, and more stable in oropharyngeal compared to nasal swab samples at all times points after infection. This data suggest that saliva may represent a more robust diagnostic sample than nasal swabs both early and later in infection.[5]

Comparison of CRISPR-FDS results for paired saliva and nasal swab samples obtained from a cohort of 31 individuals screened for COVID-19 indicated that viral loads were similar in these samples and demonstrated reasonable correlation (r= 0.8029, p<0.0001). The SARS-CoV-2 RNA levels remained stable in saliva stored at 4°C for up to 7 days after collection.[5]

CRISPR-FDS laboratory test was adapted to a chip-format assay read by a prototype smartphone-based fluorescent microscope device designed for point-of-care use, and found that the CRISPR-FDS plate reader and smartphone assays and the standard RT-qPCR assay detected similar numbers of SARS-CoV-2 positive saliva samples. Sample focusing and image acquisition were

achieved by the built-in smartphone camera app, eliminating the need for the mechanical focusing, and thus reducing weight and cost while enhancing the optical stability and user-friendliness of the device.[5] There was no information on the cost of the device obtained.

In an analysis using RT-qPCR as the reference standard, CRISPR smartphone results exhibited a 1.3% false positive rate with saliva but complete concordance with RT-qPCR results for swab samples, while CRISPR plate reader results perfectly matched RT-qPCR saliva results, but exhibited a 2.3% false negative rate with nasal swab samples. Viral load was strongly correlated in the 43 saliva samples that tested positive by both the on-chip smartphone assay and conventional RT-PCR analysis and exhibited similar mean values (3803 versus 1797 copies/μL). [5]

CONCLUSION

There was limited retrievable evidence that show good specificity and sensitivity of the smartphone-based array reader tests using saliva as sample to screen COVID-19 as well as confirming COVID-19. This technology offers short turn around time (TAT) and does not require laboratory equipment. Thus, it has a potential to rapidly expand COVID-19 screening capacity, and potentially simplify the verification of contact tracing to improve local containment.

However, further evaluation,validation and verification process with larger sample size is required to ascertain its effectiveness and safety.

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Based on available evidence up to 21th Jan 2021

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Disclaimer: This rapid assessment was prepared to provide urgent evidence-based input during COVID-19 pandemic. The report is prepared based on information available at the time of research and a limited literature. It is not a definitive statement on the safety, effectiveness or cost effectiveness of the health technology covered. Additionally, other relevant scientific findings may have been reported since completion of this report.

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