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Fluorescence-based point of care device for real-time rapid detection of SARS-CoV-2

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Summary

We report the development of fluorescence-based point-of-care device (fPOC) for rapid, on-site and at-site detection of SARS-CoV-2 using real-time reverse transcription loop-mediated isothermal amplification (RT-rLAMP). The system includes three main parts: optical setup, heating elements, and injection molded cartridge with 12 reaction chambers. By integrating the RT-LAMP on the fPOC system, the fPOC device can detect the presence of SARS-CoV-2 in clinical samples within 50 min with 99% relative accuracy, 97.5% relative specificity, and 100% relative sensitivity compared to RT-qPCR.

Keywords: point-of-care (POC), fluorescence detection, SARS-CoV-2, COVID-19, Loop-Mediated isothermal amplification (LAMP)

Background

COVID-19 caused by SARS-CoV-2 killed more than 6.9 million people and led to remarkable social turmoil and economic disruption [1]. Antigen-based quick tests have been developed and are used to detect SARS-CoV-2 in clinical samples. However, they do not reach the sensitivity required to detect the early phases of a SARS-CoV-2 infection [2-3]. Several POC devices for the detection of SARS-CoV-2 have been reported [4-6]. These POC systems had a simple design and fast detection. However, the number of samples tested per run was limited. In addition, the test cartridge was complicated and remained high cost for fabrication [7-8]. In this work, we report a fluorescence point-of-care system based on RT-LAMP technology that can overcome these drawbacks of current point-of-care systems.

Fluoresence-based Point-of-Care Device (fPOC)

The fPOC device comprises of three main parts: (1) optical setup including a light source (LED), a phototransistor and EX/EM filters; two heating elements (top and bottom heaters) to maintain the temperature (60-65^{o}C) for the rRT-LAMP reaction (Fig. 1); and (3) an injection molded cartridge with 12 reaction chambers (Fig. 2a). The cartridge was designed with special pyramid shaped optical structures located next to the reaction chambers for reflecting the LED light to the reaction chambers at 90^{0} right angle (Fig. 1). The reaction chamber is heated rapidly and maintained at the reaction temperature (60-65^{o}C) by a bottom heater and a transparent top heater. The targeted nucleic acids in the samples were amplified by RT-LAMP within the chambers and detected by SYTO-9 DNA intercalating dye. The fluorescence signals are recorded and plotted in real-time using the Parallax Data Acquisition tool (PLX-DAQ) software on an external computer. Threshold values that were used to define whether the sample was negative or positive were determined by analysing 92 nagative and 184 positive samples on fPOC. The sample was considered as positive when the fluorescence intensity was larger than the threshold value.

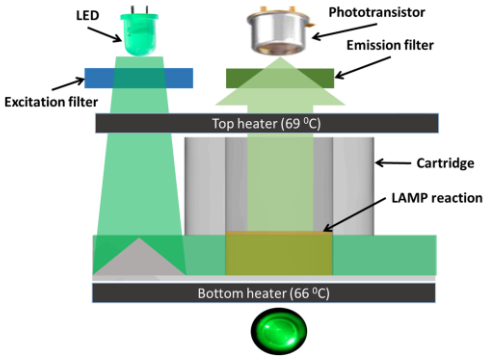

Fig. 1. fPOC working principle.

Results

The performance of fPOC was evaluated using 98 clinical samples including 58 positive and 40 negative samples confirmed by Luna® Universal One-Step RT-qPCR. Of 98 samples tested, 59 and 39 samples showed positive and negative results in the fPOC system, respectively; while 58 and 40 samples were observed positive and negative by RT-qPCR (Table 1). In comparison, fPOC showed 99% relative accuracy, 97.5% relative specificity, and 100% relative sensitivity to RT-qPCR. Moreover, the Cohens Kappa index (0.98) showed excellent agreement between these 2 methods. This result showed great potential for the use of fPOC for rapid on-site screening of SARS-CoV-2 virus in the pandemic management.

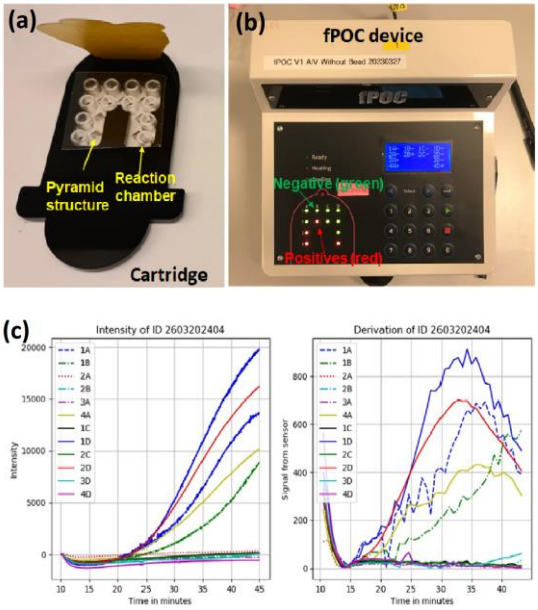

Fig. 2. (a) cartridge used in fPOC device; (b) fPOC device; (c) real-time amplification curve and derivation of amplification curve.

Table 1: Results of clinical samples tested on the fPOC and RT-qPCR

	RT-PCR			
	LOB 35	Positive	Negative	Total
fPOC	Positive	58	1	59
	Negative	0	39	39
	Total	58	40	***98***

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