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A portable nucleic acid detection system using natural convection combined with a smartphone

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ABSTRACT

The development of portable nucleic acid diagnostic devices has the potential to expand the availability of molecular diagnostics into low-resource settings. One of the promising solutions for rapid and simple DNA amplification is the use of Rayleigh–Bernard natural convection which is caused by a buoyancy-driven thermal gradient of liquid when heated from below. This natural convection avoids the use of the complex and sophisticated hardware that is required for precise maintenance of temperature cycles in conventional PCR. We have developed a stand-alone convective PCR (cPCR) device linked to a smartphone for rapid detection of nucleic acids using natural convection heating. The device amplifies multiple DNA samples simultaneously using a custom-made heat block controlled by Bluetooth wireless communication. The entire device is highly portable, user-friendly, battery-operated and can provide target DNA amplification in less than 30 min. A detection limit of 2.8×10^3 copies of a segment of lambda DNA was obtained when the two different fluorescently-tagged amplicons were collected magnetically and detected using the smartphone fluorescence reader. Thus, the combination of cPCR and multiplex fluorescence-based detection on a smartphone provides new opportunities for the development of affordable and portable molecular diagnostic devices for point-of-care situations or remote clinical settings.

1. Introduction

PCR and related techniques have revolutionised the field of molecular diagnostics for the detection of viral, bacterial and parasitic infections. The technique allows quantification of a target sequence which directly relates to the quantity of the infecting agent. Real-time PCR also can be multiplexed, allowing simultaneous amplification of several target genes, offering a sensitive alternative to traditional immunoassays and culture techniques (Wang and Salazar, 2016). In PCR-based detection, thermocycling is performed by repeated heating and cooling of the sample, which requires time and a source of energy to regulate and maintain the necessary temperature cycles. The sophisticated hardware and the reaction time for turnaround required to perform repeated temperature cycles for PCR have been the main focus of several innovations to simplify this technology.

Farrar and Wittwer (2015) demonstrated that a super-fast PCR reaction with 20-fold increased primer and polymerase concentrations, increased annealing/extension temperatures (75 °C) and reduced denaturation temperatures (90 °C) allow amplification in 15–60 s. However, super-fast PCR is suitable only for clinical laboratories and requires skilled personnel preventing its possible point-of-care (POC) diagnostic capabilities. Tay et al. (2016) recently developed a continuous-flow PCR that uses microfluidic channels to move samples through various temperature zones, thus reducing the time required for amplification. Recent developments include droplet-based microfluidic PCR, which is claimed to be ideal for single-cell and single-molecule analyses and is claimed to show potential for system integration and automation (Zhu et al., 2012). Most PCR devices commercially-available use dedicated chips and cartridges that can provide sample-to-answer detection of pathogens (Kwon et al., 2015; Nair et al., 2016). Although promising results have been obtained, the complexity associated with their operation requires external fluidic pumps (Zhang and Jiang, 2016) imposing a high requirement on chip fabrication, increasing cost and bulkiness and making it difficult to provide for POC

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settings.

A portable and affordable instrument for nucleic acid detection could make a significant difference in the accessibility of molecular diagnostics in real world settings.

One promising solution for simple DNA amplification is the use of Rayleigh – Bernard natural convection, where the DNA template is amplified by cycling between hot and cold regions via a buoyancy-driven flow of liquid when heated from below (Chou et al., 2011). The reaction mixture establishes a convective flow when placed on a heat block maintained at 95 °C, which drives the sample through the temperature zones associated with denaturation, annealing and extension stages of PCR. 35–40 cycles of PCR can be performed in less than 30 min. This approach drastically reduces the need for electrical power, cost and time required for amplification of target DNA as used in conventional PCR machines (Krishnan et al., 2002). These features have provided convective PCR (cPCR) with a promising role in the implementation of POC molecular diagnostics. In the last decade, several variants of cPCR-based devices have been reported including those utilising simple micro-immersion heaters, solar thermal energy and thermally baffled devices (Priye and Ugaz, 2016). The glass capillary has been the most acceptable reaction chamber but recently polycarbonate rod and pre-loading of dry reagents were introduced in cPCR (Priye et al., 2016; Qiu et al., 2017b). Usually, the amplicons obtained by cPCR are detected by agarose gel electrophoresis, electrochemistry, fluorimeter and nucleic acid-based immunochromatographic assays (Phaneuf et al., 2015; Zhang et al., 2014). Despite the possibilities provided by these detection methods, the challenge remains of incorporating cPCR amplification and detection of amplicons into a single, simple, portable and POC format. Recently, smartphones have been increasingly significant in POC diagnostics owing to their simple optical attachment to enable fluorescent detection of nucleic acids. Several analytical systems have been developed that employ smartphone cameras for fluorescence and colorimetric imaging followed by quantification of nucleic acids and proteins through a remote server (Berg et al., 2015; Kim et al., 2018).

We report here a smartphone-based thermal convective device that combines the distinct advantages of cPCR and the smartphone for multiplex amplification of DNA that is controlled by simple hardware and is communicated wirelessly via Bluetooth technology. The amplification of DNA by cPCR is combined with a simple magnetic capture and enrichment step to provide a sensitive and multiplex fluorescence-based detection of nucleic acids (Scheme 1). As a proof-of-concept demonstration, two fluorescent dyes were used as labels for multiplex detection of cPCR amplicons using the smartphone reader. The results presented suggest that the proposed smartphone-integrated device provides a robust tool for POC diagnostic applications.

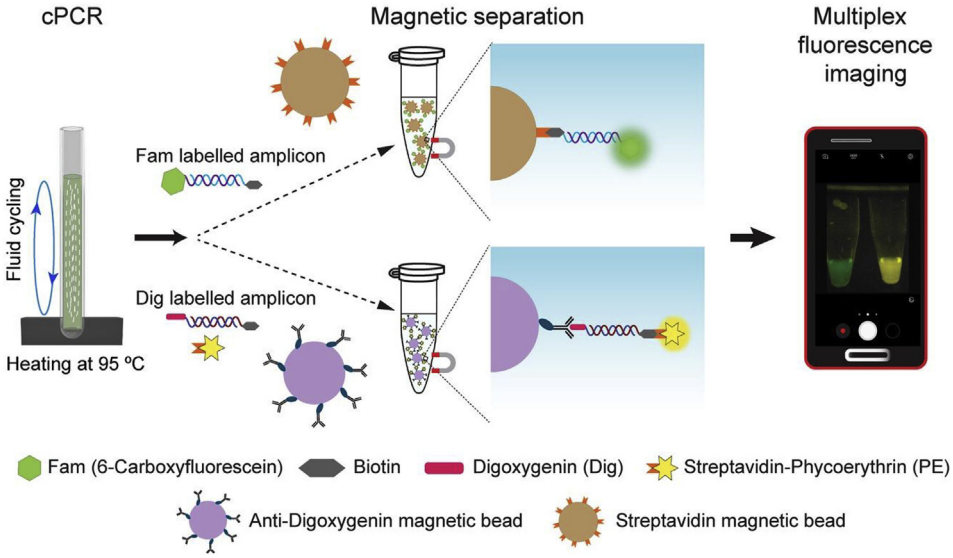
2. Experimental

2.1. Design of cPCR device

The heating unit was constructed using a custom-made machined aluminium block containing the appropriate chambers for accommodating the cPCR capillary tubes (Mechanical Workshop, School of Chemistry, University of New South Wales, Sydney, Australia). The thermal block was designed using computer-aided design (CAD) software (Autodesk 123D and Autodesk Fusion 360). The aluminium block consisted of four holes in the top for placing the capillaries and a single hole at the side to house the temperature sensor (Fig. S1). A Peltier element (Multicomp element14, Australia) was attached to the aluminium block using heat sink thermal tape (8810, 3M™, USA). The temperature sensor (LM35DZ, Texas Instruments, USA) was attached to the side hole in the aluminium block for feedback control. The Peltier element was controlled using a DRV8835 dual motor driver (Pololu Corporation, USA) with the controlling signal from Arduino nano-microcontroller (Arduino, Italy). The temperature was controlled using a LM35DZ sensor connected to the microcontroller. The set temperature of 95 °C with resolution of ± 2 °C was reached within 2–3 min and maintained consistently for the total reaction time of 30 min (Fig. S1). All the units were powered by a 3.7v lithium ion battery (Master Instruments, Australia). The input for the functioning of the various components of the device was provided and controlled using the custom-developed android application *Intensity* (Fig. S2) running on the smartphone (Google Nexus, model 5X with 12.3 mega pixel CMOS image sensor, 1.55 µm pixel size and aperture size of f/2.0) via Bluetooth communication.

2.2. cPCR assay

Commercially-obtained lambda DNA (500µg/mL, New England Biolabs Ltd, USA) was used for optimisation of the cPCR to obtain two amplicons of 84 bp and 175 bp in size. Primers were designed using the Primer 3 software targeting the lambda DNA sequence (Untergasser et al., 2012). The two forward primers consisted of 5′ 6-carboxy



Scheme 1. Schematic representation of the magnetic bead capture detection strategy for cPCR amplicons. The target DNAs were amplified independently using FAM and streptavidin-PE followed by magnetic capture and smartphone fluorescence imaging and quantification.

fluorescein (FAM) AATCAGCCGGCGATGCCAGTGCATCAGCT 3′ and 5′ digoxigenin CGGCCGGAGGTGCTGCGTCGT and were designed with one common reverse primer of 5' biotin TGCTGGCGGCGCGTGCTGAGT 3'. All primers were synthesised by Integrated DNA Technologies (Singapore). The cPCR assay was performed in custom-designed glass capillaries with an internal diameter of 1.6 mm, an outer diameter of 3 mm and a height of 35 mm. The capillaries were washed in acetone and ethanol using an ultrasonic cleaner (Transducer power 250 W and 40 KHz frequency, UNISONICS, Australia) followed by several rounds of ultrapure water and autoclaving before use. The reaction mixture for the cPCR assay consisted of 10 μL of 5 x MyTaq master mix (Bioline Pty Ltd, Sydney, Australia), 2.5 μL each of 10 μM of FAM-labelled forward primer/digoxigenin forward primer and biotin labelled reverse primer, 0.75 μL of 5 Units/μL (3.75 Units/50 μL reaction volume) MyTaq DNA polymerase, 3 μL (50 ng) of lambda DNA and 31.25 μL of nuclease free water to make the final volume to 50 μL. The capillary tubes containing 50 μL of reaction mixture were overlaid with 10 μL of mineral oil before conducting the cPCR assay and placed into the aluminium heat block maintained at 95 °C for 30 min for amplification. Lambda DNA (50 ng/μL) was diluted serially (1:10 steps to obtain 5–0.0005 ng/μL of lambda DNA) and used to determine the minimal detection limit. A sample without the lambda DNA was used as control to demonstrate the specificity of the cPCR assay. The amplified products were transferred from the capillary tubes to 200 μL microcentrifuge tubes and stored at −20 °C for further analysis by the multiplex magnetic bead capture assay.

2.3. Preparation of magnetic beads

Two types of magnetic beads were used for enrichment and separation of the fluorescently-labelled cPCR amplicons, enabling the specific capture of FAM and PE labelled cPCR amplicons by binding either to biotin or digoxigenin labels. First, 200 μL of Sure Beads™ Protein G Magnetic beads (10 mg/mL, Bio-Rad Laboratories, USA) was washed magnetically three times with 100 mM Tris HCl buffer containing 0.1% Tween 20, pH 7.5. Then 10 μL of Pierce anti-digoxygenin sheep polyclonal antibody (5 mg/mL, Life Technologies, Australia) was diluted to 200 μL in 50 mM Tris HCl buffer, pH 7.5 and added to the Protein G magnetic beads. The mixture was incubated at room temperature in a rotary mixer for 15 min and the beads washed magnetically three times with 100 mM Tris HCl buffer containing 0.1% Tween 20, pH 7.5 and the supernatants discarded using a custom 3D printed magnetic rack (Fig. S3). The final pellet was reconstituted in 200 μL of 50 mM Tris HCl buffer, pH 7.5 and used for magnetic separation of cPCR amplicons. The mixture will be referred to as anti-digoxigenin magnetic beads in this account.

Secondly, 500 μL of Sphero™ streptavidin magnetic beads (0.5% w/v, 2.69 μm, Spherotech, USA) was washed three times in 100 mM Tris HCl buffer containing 0.1% Tween 20, pH 7.5 and the final pellet was resuspended in 250 μL of 50 mM Tris HCl buffer, pH 7.5 and stored at 4 °C until further use for agarose gel electrophoresis and magnetic separation of biotin-modified cPCR amplicons.

2.4. Magnetic bead capture assay

The lambda DNA target was amplified by cPCR separately and the two different amplicons (84 and 175 bp) were detected using FAM and streptavidin-labelled phycoerythrin (streptavidin-PE) by the magnetic bead capture assay as shown in Scheme 1. After cPCR, 10 μL of the 84 bp target amplified using FAM-labelled forward primer and biotinylated reverse primers was mixed with 30 μL of Sphero™ streptavidin magnetic beads and the volume was made up to 50 μL with 50 mM Tris HCl buffer, pH 7.5 and incubated at room temperature for 30 min. Samples were washed as before and finally resuspended in 30 μL of the same buffer and used for fluorescence imaging with the smartphone reader. The amount of cPCR amplicon required for the magnetic bead capture assay was determined by using various volumes (2.5, 5, 7.5 and 10 μL) of cPCR amplicons (Fig. S4).

Similarly, the 175 bp cPCR product amplified using digoxigenin forward primer and biotin reverse primer was used for the magnetic bead capture assay. First, 10 μL of eBioscience™ streptavidin PE (0.2 mg/mL, Thermo Fisher Scientific, Australia) was diluted to 100 μL using 50 mM Tris HCl buffer, pH 7 and 10 μL of diluted streptavidin PE and 20 μL of anti-digoxigenin magnetic beads was added to 10 μL of cPCR product. The final volume was made up to 50 μL with 50 mM Tris HCl buffer (pH 7.5) and the samples were incubated in a rotary mixer for 10 min at room temperature after which the mixture was washed as before and finally resuspended in 30 μL of the same buffer and used for fluorescent imaging. The detection limit of the magnetic capture was determined using the cPCR amplicons obtained from amplification of serially-diluted lambda DNA as a template for both methods.

2.5. Smartphone image acquisition and analysis

A simple optical design was printed using PLA (polylactic acid)/ABS (acrylonitrile butadiene styrene) filament on 3D printer (XYZ DaVinci 1.0A 3D printer; XYZPrinting Inc) and attached to the smartphone for detection of the magnetic bead capture assay. The device was adapted from our earlier study (Rajendran et al., 2014) with slight modifications and consisted of a 3.7 V battery-powered blue LED light source with a dominant wavelength at 470 nm with luminous flux of 45 lm (Osram Opto Semiconductors) for illumination, a fluorescence dichroic mirror for FAM excitation (Chroma Technology Corp, USA) and a plano convex lens (Edmund Optics, USA) of 30 mm focal length (Fig. S5). The band pass filter (452 nm ± 45 nm, Edmund Optics) in the filter set allowed the excitation of the fluorophore and eliminated the high wavelength tail of the LED emission. The emission filter SYBR™ Photographic filter (Thermo Fisher Scientific) enabled simultaneous observation of multicolour fluorescent signals from the samples. After magnetic capture of the fluorescent signal, the optical components were enclosed into a 3D printed module (Fig. S5) and attached in front of the 12.3-megapixel camera of the Google Nexus 5X smartphone. The fluorescent images captured were analysed using the ImageJ mobile app (IJ Mobile downloaded from Google Play Store developed by Dr. Michael Steptoe, VADER lab, Arizona State University) running on the smartphone to determine the fluorescent intensity after magnetic capture. The images were aligned with Image Combiner app (Google Play Store) and imported to the ImageJ app to calculate the mean pixel intensity across all the sample tubes. The calculated intensity was displayed as a graphical output where the peak intensity varies with fluorescent intensity related to the concentration of target in the test sample.

2.6. Sensitivity assay of smartphone reader

The sensitivity was determined using the various dilutions of FAM-labelled probe DNA and streptavidin-PE in 50 mM Tris HCl buffer, pH 7.5. The assay was performed by serially diluting 10 μM of FAM-labelled ssDNA (between 10 and 0.03 μM) and imaging the tubes using the smartphone reader. Similarly, streptavidin-PE was diluted serially (between 42 and 0.16 nM) and imaged. Three individual experiments were performed to determine the detection sensitivity of the smartphone reader and the results compared to the detection obtained using a PHERAstar (BMG Labtech, Ortenberg, Germany) fluorescence plate reader (Fig. S6).

3. Results

3.1. Portable cPCR device

Our goal was to develop an affordable smartphone-integrated cPCR instrument for multiplex detection that could be assembled easily from 3D-printed parts and off-the-shelf electronic components. Fig. 1A is the

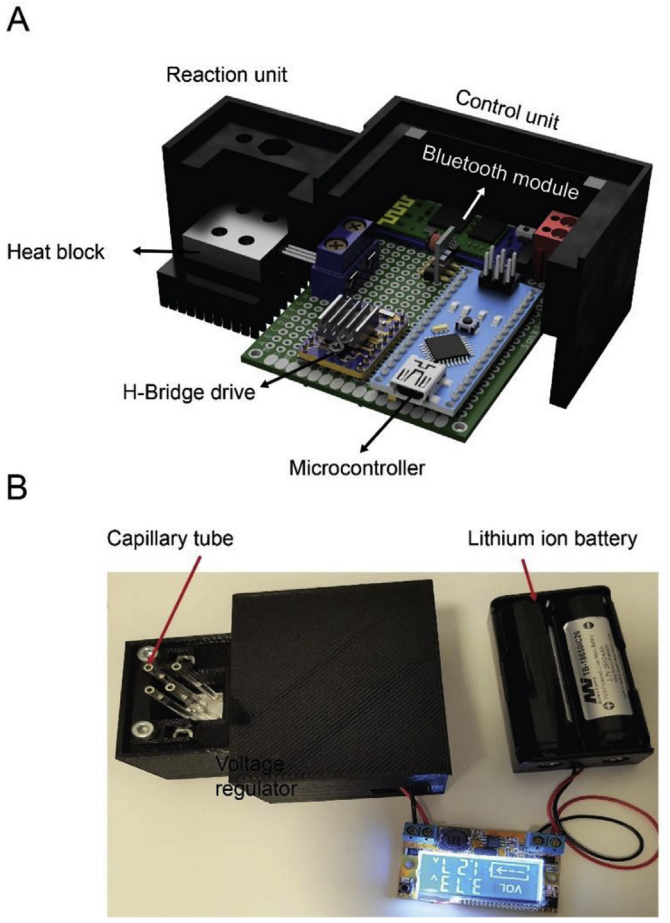


Fig. 1. Assembly of the different components of the cPCR. (A) 3-D model made using Autodesk Fusion 360 shows the inner view of the different parts of the cPCR device which consist of the control unit and reaction unit to hold the capillaries above the heat block. (B) Final assembled cPCR device housed inside the 3D printed chassis holding four capillary tubes.

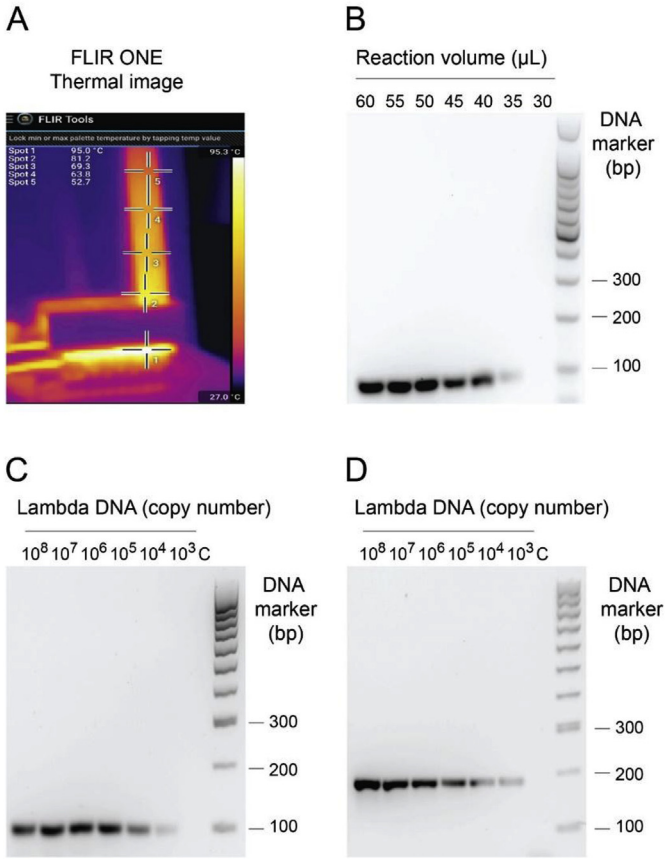


Fig. 2. Evaluation of the cPCR device. (A) FLIR ONE thermal imaging of cPCR capillaries obtained during the reaction showing the temperature zones associated with denaturation, annealing and extension stages. (B) cPCR amplification using different reaction volumes per capillary. (C–D) Detection limit of cPCR after serially-diluting 2.8×10^8 copies of lambda DNA. Two amplicon, 87 bp and 175 bp, were targeted by the specific primers. C; negative control sample without template lambda DNA.

cross-sectional view of the cPCR device showing the inner components before their final assembly into a 3D-printed, enclosed device. The device consisted of a control unit, reaction unit and battery (Fig. S7). The control unit included all the electronics that control the device as well as Bluetooth communication. The reaction chamber consisted of the Peltier module with the heat block holding the reaction capillaries. We used a low-cost Peltier unit to maintain the high temperature required (95 °C) for cPCR. The H-bridge driver was used to drive the Peltier module as it is a high-power device which the microcontroller cannot handle alone.

The feedback signal from the temperature sensor attached to the side of the heat block was used to control the temperature during the reaction. The heat block was designed to accommodate four reaction capillaries simultaneously and a fastened lid provide a tight contact. Two lithium ion batteries were used to power the device. All components, including the control unit and reaction unit were assembled and packed into a 3D-printed module with dimensions of 100 mm (L) x 80 mm (W) x 40 mm (H) (Fig. 1B). Finally, the parameters required for controlling the temperature and duration of the reaction were provided by the smartphone using the *Intensity* app through a USB or Bluetooth interface. This app provided a simple interface for easy configuration and control of the cPCR device as well as the smartphone at remote locations.

3.2. Evaluation of the cPCR device

The design of the cPCR device was verified by evaluating its temperature control and the thermal gradient generated. Thermal images were obtained during the cPCR reaction with a FLIR ONE thermal imaging camera. The Peltier module was used to provide the temperature of 95 °C required for generation of convective flow and also provided uniform heating to all four reaction capillaries to allow multiple sample amplification. Initially, the reaction mixture was placed in the capillaries and kept at 95 °C on the cPCR device. Thermal images of the capillaries were processed using FLIR tools android application to analyse the temperature gradient across them. Thermal images (Fig. 2A) show the different temperature profiles across the capillaries when subjected to the cPCR reaction. Spot 1 in the image represents the heat block maintained at 95 °C. Subsequently, different temperatures were observed across the capillaries showing 81 °C, 69 °C, 63 °C and 53 °C at the spots 2–5 of the capillaries. Thus, the respective temperatures required for the denaturation, annealing and extension were maintained along the length of the capillaries, demonstrating the successful generation of convective flow. The successful amplification of lambda DNA was demonstrated also at various environmental temperatures (4, 23 and 37 °C) and allowed confirmation that our system could operate consistently across different temperature conditions (Fig. S8).

The most suitable reaction volume was determined for optimal lambda DNA amplification. Successful amplification was observed at

different reaction volumes (30–60 µL) with an optimal volume around 50 µL as indicated by the intensity of the product band observed after agarose gel electrophoresis (Fig. 2B). Further cPCR assays were performed using a 50 µL reaction volume at 95 °C for 30 min.

The versatility of cPCR was demonstrated by amplification of two different targets for lambda DNA in the device. Serially-diluted lambda DNA (10^8-10^3 copies) was used as template for cPCR and amplified to obtain two amplicons of 84 and 175 bp. As observed in Fig. 2C and D, successful amplification of the 84 bp and 175 bp product was observed until 50 fg/µL of DNA was reached, which was equivalent to 2.8×10^3 copies of lambda DNA. In addition, the cPCR device was validated for amplification of various gene target on a synthetic DNA construction to demonstrate the multiplex amplification of four target regions in a single tube (Fig. S9). The simultaneous amplification of four gene targets consisting of polymerase gene (115 bp) of hepatitis B virus, haemagglutinin gene (151 bp) and matrix gene (176 bp) of Influenza virus, methicillin resistant gene (219 bp) of *Staphylococcus aureus* in a single reaction shows the capability of the cPCR device to amplify various amplicon lengths and multiplex potentials. Also, the cPCR assay was performed using genomic DNA obtained from a clinical isolate of methicillin resistant *S. aureus* targeting the *femA* gene and used for the magnetic bead-based capture assay (Fig. S10). The successful amplification and detection of *S. aureus* DNA shows the potential for routine diagnostic application to clinical isolates.

3.3. Characterisation of the smartphone fluorescence reader

One of the challenges of POC molecular diagnostic devices is the incorporation of simple fluorescence detection platforms which do not require sophisticated instrumentation. We have developed a fluorescence reader that leverages the rapidity of cPCR and versatility of the smartphone to enable multiplex in-field diagnostics. Accordingly, we used all components of the android smartphone including display, camera, processor and Wi-Fi/Bluetooth connectivity to achieve laboratory-quality fluorescent detection of amplicons. The fluorescence reader consisted of a cPCR tube holder and an optical module (Fig. S5) attached to the camera of the smartphone (Fig. 3A) using 3D-printed parts.

The sample tubes in the tube holder were inserted into the slot provided in the optical module attached to the smartphone that can accommodate up to three tubes for simultaneous imaging (Fig. 3B). Upon excitation with the LED, the fluorescence from the sample tubes passed through the optical module in the epifluorescence mode (Fig. S5) and was imaged using the smartphone camera as shown in Fig. 3C. The configuration of the fluorescence reader enabled simultaneous imaging of FAM and PE dyes. The images obtained were processed to allow analysis of the fluorescent intensity signal from the magnetically-captured cPCR amplicons using the IJ_Mobile app running on the smartphone.

Initially, the smartphone reader was calibrated for detection sensitivity using serially-diluted FAM-labelled DNA (Fig. 4A) and streptavidin-PE (Fig. 4B). Successful fluorescent signals were imaged down to a concentration of 0.07 µM of FAM-labelled DNA and 1.31 nM of streptavidin-PE. Further quantification of the images by the smartphone 'app' shows the decrease in fluorescent intensity proportional to the concentration of the fluorescent dye (Fig. 4C and D). This sensitivity assay was used to establish the dynamic range for the device and to demonstrate that parallel detection of two fluorescent dyes using the reader was possible.

3.4. Smartphone-based detection of the products of cPCR by magnetic separation

Instrument-free post amplification processing of cPCR could reduce the time and cost required for quantitative detection of nucleic acid amplicons. We introduced the magnetic bead capture of cPCR amplicons to allow multiplex fluorescent detection with increased sensitivity. Magnetically-captured, fluorescently-labelled amplicons were detected using the smartphone with the provision of quantitative detection displayed through a dedicated 'app'. The assays containing various concentrations of target DNA were amplified using FAM-labelled and digoxigenin-labelled primers with the common biotin reverse primer in parallel reactions for approximately 30 min and captured magnetically. The fluorescently-labelled amplicons were excited using a LED and detected using the smartphone reader. The fluorescent images from the sample tubes were captured and provided the qualitative yes/no detection of cPCR by naked eye. The images captured were imported into the ImageJ application to evaluate the intensity with the concentration of the target.

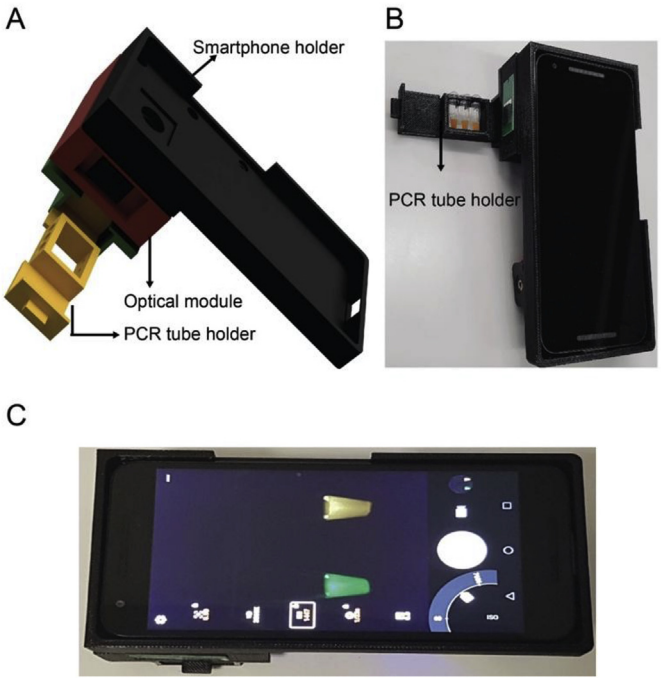


Fig. 3. Smartphone fluorescence reader. (A) 3D model showing the PCR tube holder, optical module and smartphone cradle, (B) the assembled fluorescence reader showing the sample holder inserted into the reader, (C) the final assembled smartphone reader showing the multiplex fluorescence detection of the tubes after magnetic bead capture assay.

The plot profile was analysed to show the respective fluorescent intensity of FAM (Fig. 5A) and PE (Fig. 5B) across the tubes. The fluorescent intensity was recorded after magnetic capture of the cPCR amplicons representing 2.8×10^3 copies of 84 bp and 175 bp lambda DNA. The readings from five individual experiments were plotted to allow observation of the detection sensitivity and reproducibility of the smartphone reader (Fig. 5C and D). In total, the smartphone fluorescent reader developed here provides instant quantification and multiplex detection of cPCR amplicons targeting different regions of lambda DNA.

4. Discussion

POC diagnostics has received increased attention in recent years due to its perceived advantages over laboratory-based tests. They include portability, reduced cost and increased user-friendliness (Chan et al., 2018). Currently, smartphone-based systems have been reported that provide fluorescence, electrochemical and colorimetric detection of biological molecules (Cmiel et al., 2016; Mutlu et al., 2017; Qiu et al., 2017a). The ubiquitous camera found in current smartphones is an ideal tool that can be exploited for biosensing applications. A smartphone reader coupled with a portable cPCR device was developed in this study to capture and process the signal from analytes and allow

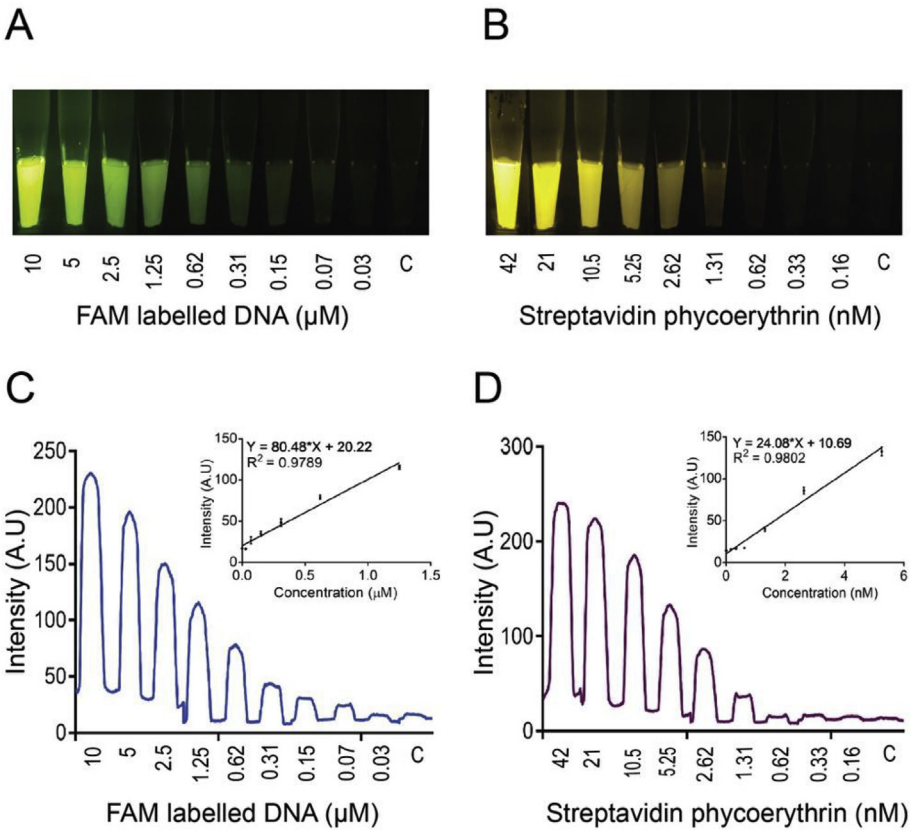


Fig. 4. Smartphone fluorescence reader for detection of the image obtained from various dilutions of (A) FAM-labelled DNA and B) streptavidin-PE. Fluorescent intensity of the image after processing using the ImageJ application on the smartphone for (C) FAM and (D) PE. C; negative control sample without fluorescent dyes. Insets to C and D show the linear regression fit of the fluorescent intensity with respect to increasing concentrations of fluorescent dye. Error bars were obtained from repeated experiments (n = 3).

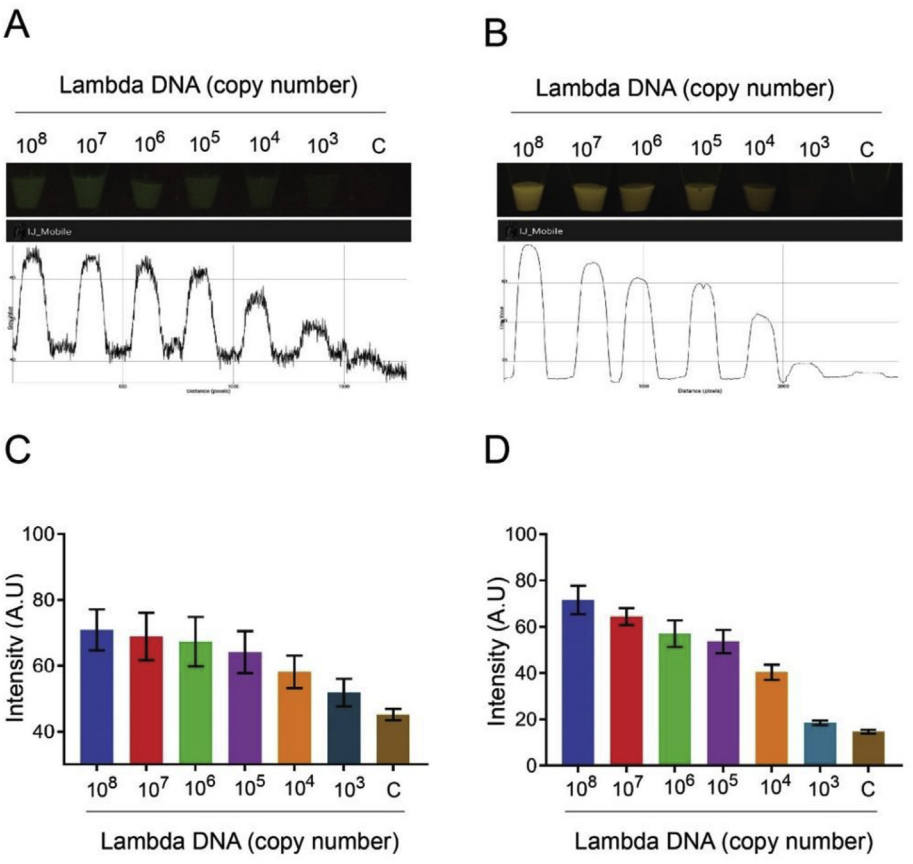


Fig. 5. Multiplex detection of cPCR by magnetic bead capture assay using FAM and streptavidin-PE and the smartphone fluorescence reader. Image obtained after serially diluting 2.8×10^8 copies of lambda DNA amplified by cPCR and the corresponding intensity profile obtained by ImageJ application on the smartphone for (A) FAM and (B) PE. The graphs show the detection limit of cPCR measured after using the smartphone reader with error bars obtained from repeated (n = 5) experiments for (C) FAM and (D) PE. C; negative control sample without template lambda DNA.

Table 1
Comparison of available cPCR devices on a smartphone reader platform.

Heating unit	Smartphone integration			Multiplex detection	Detection limit	Ref
	Device control	Detection	Analysis			
Multiple heater	•	•	•		10^3 copies/μL[a]	Priye and Ugaz (2017)
Exothermic reaction		•			1 $TCID_{50}$/mL [b]	Qiu et al. (2017c)
Resistive heater		•	•		1 $TCID_{50}$/mL	Qiu et al. (2017a)
Ceramic resistor		•	•		10^3 copies/μL[a]	Priye et al. (2016)
Single Peltier unit	•	•	•	•	10^3 copies/μL[a]	This study

[a] copies of lambda DNA.
[b] $TCID_{50}$/mL – 50% tissue culture infective dose.

communication via USB port or Bluetooth. Priye et al. (2016) demonstrated the flexibility of this technology by developing a cPCR amplification system for rapid field deployment using quadcopter drone. They utilised the smartphone for fluorescence detection either in an end-point mode or in a time-resolved manner enabling reaction progress to be monitored continuously with a detection limit of 10^3 copies/μL of lambda DNA. Further developments by the same group resulted in a portable device where the smartphone was used for device control, fluorescence detection and an 'app' for analysis of test results from 10^3 copies of DNA of lambda DNA amplified by the cPCR assay (Priye and Ugaz, 2016). Qiu et al. (2017a,c) reported two independent approaches using TaqMan probes and lateral flow strips for detection of cPCR amplicons using the camera of the smartphone as a sensor for detection of H1N1 virus for real-time and end-point fluorescence detection respectively. Comparison of the performance, portability and multiplexing capability between our device and other portable cPCR devices incorporated into a smartphone platform is presented in Table 1.

Despite their portability, none of the cPCR approaches available have been reported to have multiplex capability on a smartphone platform. To simplify the multiplex detection procedure, we incorporated a magnetic bead-based separation step into our platform to allow for fluorescence detection of more than one cPCR amplicons. Multiplex assay capability was achieved with a detection limit of 10^3 (equivalent to 2.8×10^3) copies of lambda DNA which is equivalent to the sensitivity of current-available single cPCR detection approaches relying on an intercalating dye to monitor amplification.

Recently, the open-source platform and tunable hardware Arduino was implemented successfully as a tool to execute several tasks including control of temperature and microfluidic systems for sample movement during the prototype device development (Chen et al., 2014; Mulberry et al., 2017). The wide compatibility of the Arduino module made it easy to integrate the smartphone for the control of the portable cPCR device (Priye et al., 2016). The operation was maintained using an Arduino microcontroller through a specific program written using Arduino IDE. The open-source electronic prototyping platform enabled us to reduce the total cost of our cPCR device to less than US$ 100 by using low-cost, off the shelf electronic components. This sum represents a 10 times cost reduction when compared to the use of immersion heaters and an infrared laser for heating (Braun et al., 2003; Hennig and Braun, 2005). The cPCR device using a ceramic heater by Priye et al. (2016) reduced the cost of the device further to US$ 50, but it is not suitable for multiple sample amplification. The portable PCR instruments currently available rely on expensive microfabrication techniques like micromachined silicon heaters to produce the heating block of the thermocycler (Ahrberg et al., 2016a, b). In this current format, the use of a single Peltier unit enables four simultaneous amplification reactions to take place, making the device much cheaper than commercial real-time PCR instruments (US$ 20,000) and other real-time cPCR devices (US$ 1000) (Derendinger et al., 2018; Hsieh et al., 2013).

Quantitative analysis of the amplified products relies heavily on expensive optical detection components though the cPCR approach simplifies and reduces the cost of the thermal cycling instrumentation. A typical detection unit of portable devices incorporates an expensive photodiode, charge-coupled device and photomultiplier tube to enable fluorescence quantification (Hsieh et al., 2013; Mauk et al., 2017; Xin et al., 2012). The fluorescent signal from these detectors must be sent to a computer for further processing and analyte detection. For example, the benchtop POCKIT system (GeneReach) is a commercial cPCR instrument which lacks both an integrated battery and a detection system, and thus requires post-amplification processing of the amplicons by agarose gel electrophoresis (Agrawal et al., 2007). Another commercially-available capillary convective-based device, the Palm PCR instrument (Ahram Biosystems), offers battery power but also requires post-amplification processing for detection of nucleic acids, increasing the time and cost of the assay.

5. Conclusions

We describe a stand-alone convective PCR (cPCR) device linked by Bluetooth to a smartphone for rapid detection of nucleic acids. The device was able to amplify multiple DNA samples and is highly portable, user-friendly, battery-operated and inexpensive. It can provide target DNA amplification in less than 30 min with a detection limit of 2.8×10^3 copies of a selected segment of lambda DNA obtained when fluorescently-tagged amplicons were concentrated magnetically and quantified using a fluorescence reader based on a smartphone. A major limitation of the current device is the lack of an integrated sample preparation capability. The development of an automated microfluidic platform would allow performance of a complete 'sample to answer' assay and would be a significant advance for resource-limited settings. Future developments include enhanced sample processing and expansion for the detection of wider panel of nucleic acid targets.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CRediT authorship contribution statement

Vinoth Kumar Rajendran: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing - original draft, Writing - review & editing. **Padmavathy Bakthavathsalam:** Conceptualization, Methodology, Writing - original draft. **Peter L. Bergquist:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision. **Anwar Sunna:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.03.050.

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