A low-cost PCR instrument for molecular disease diagnostics based on customized printed circuit board heaters

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Abstract

This article describes the fabrication of a low-cost Polymerase Chain Reaction (PCR) instrument to detect diseases. In order to reduce the instrument price and simplify construction we developed an alternative fabrication process, transforming conventional printed circuit boards (PCB) in heating elements, avoiding the use of aluminum heating/cooling blocks and Peltier devices. To cool down the reaction a simple computer fan was used. The vial holder was fabricated using two double side PCB boards assembled in a sandwich-like configuration. The bottom PCB has a resistance of 0.9 Ω used to heat the reaction mix, while the top layer has a resistance of 1.1 Ω to heat the vial body, preventing vapor condensation. The top board was maintained at ~110 ± 1 °C during all cycles. The final device was able to heat and cool down the reaction at rates of ~2.0 °C/s, a rate comparable to commercial thermocyclers. An SMD NTC thermistor was used as temperature sensors, and a PID (proportional–integral–derivative) control algorithm was implemented to acquire and precisely control the temperature. We also discuss how the instrument is calibrated. The device was tested successfully for the amplification of *T. pallidum* (Syphilis) bacterial DNA and Zika virus RNA samples, showing similar performance to a commercial PCR instrument.

1 Introduction

Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify fragments of DNA in vitro (Chiou et al. 2001; Erlich 2015; Innis et al. 2012; Mullis et al. 1986; Mullis and Faloona 1987; Xianbo and Jingqi 2005) using a mixture of reagents, such as: master mix, DNA (target to be amplified), oligonucleotide primers, polymerase enzyme and dNTPs (deoxynucleotides) (Zhang et al. 2006). This biochemical reaction occurs heating and cooling down the targeted nucleic acid and the molecular reagents in cycles (Chiou et al. 2001; Innis et al. 2012; Xianbo and Jingqi 2005). These cycles occur in three main steps; DNA denaturation (~ 90—95 °C), annealing of primers (~ 55—70 °C) and extension (~ 60—72 °C) (Chiou et al. 2001; Erlich 2015; Gibbs 1990; Innis et al. 2012; Xianbo and Jingqi 2005; Zhang et al. 2006). When amplifying an RNA molecule, an additional step is necessary to convert RNA into a cDNA molecule, using the reverse transcriptase enzyme (RT). The reverse transcriptase—polymerase chain reaction (RT-PCR) has been proved to be a rapid and sensitive method to detect nucleic acids from related viruses in clinical material (Belák 2005; Fernández et al. 2008; Huang et al. 2001; Kuno 1998), including arbovirus like yellow fever (Dash et al. 2012), Zika vírus (Faye et al. 2013, 2008) and dengue (De Castro et al. 2004; Naze et al. 2009). In general, optimal RT enzyme activity and maximum cDNA length occur at 42–48 °C, but this reaction temperature can range from 25 °C to 58 °C (Gerard et al. 2002). As can be seen, each step in a PCR or RT-PCR reaction occurs under optimized temperature conditions, therefore the instrument used to promote the amplification needs to be precise (Erlich 2015; Miralles et al. 2013). The use of PCR devices is today the most reliable method to identify bacteria and viruses (Clementi et al. 1993). Conventional PCR instruments generally use a solid-state device called Peltier to cool down and heat up an aluminium block (Xianbo and Jingqi 2005; Zhang et al. 2006) that supports 200 μL conical vials containing the samples, but generally only 20–50 μL of liquid is used on it. Therefore, conventional instruments waste energy to heat a large aluminium block unnecessarily. As the denaturation

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of DNA occurs at temperatures near 95 °C, evaporation becomes a concern (McPherson and Møller 2006; Yoshihiro 2003), and to prevent the condensation of liquid on the vial cap, PCR instruments have a heated lid to heat the vial cap to ~ 105 °C (McPherson and Møller 2006). Considering the precise temperature control in PCR devices is essential (Miralles et al. 2013; Xianbo and Jingqi 2005), the simple turn ON/OFF from the heater and cooling elements when the desired temperature is achieved is not enough. The most widely used technology to control the temperature with precision is using PID (proportional–integral–derivative) algorithms (Ang et al. 2005; Åström and Hägglund 1995; Johnson and Moradi 2006; Kushwah and Patra 2014; Visioli 1999). A PID controller is a control loop feedback mechanism widely used in industrial control systems and a variety of other applications (Ang et al. 2005; Dinca et al. 2009), including PCR instruments (Dinca et al. 2009; Pogfai et al. 2008; Xianbo and Jingqi 2005). PID control consists of a loop system in which the error measured is used as a source to control actions that will be implemented via the PID. There are three main parameters responsible for the control: the proportional action Kp, the integral action Ki and the derivative action Kd (Ang et al. 2005; Åström and Hägglund 1995). On PID systems, the temperature sensor constantly feeds the system and the electronic device continuously calculates the error value as the difference between a desired setpoint and the measured temperature, and applies a correction based on the PID Eq. (1) (Johnson and Moradi 2006; Visioli 1999).

$$u(t) = Kp.e(t) + Kd.\frac{de(t)}{dt} + Ki.\int_0^t e(\tau)\,dt \qquad (1)$$

PID equation: Where $u(t)$ is the control variable, $e(t)$ is the control error, Kp is the proportional gain, Kd is the derivative gain and Ki is the integral gain.

The tuning method is heuristic and based on the Ziegler-Nichols method, which consists in obtaining the ultimate gain of the circuit and using this information to set the Kp, Kd and Ki gains (Kushwah and Patra 2014; McAvoy 1972; Ziegler and Nichols 1993). Likely, there are open-source libraries that contain all elements present in the PID equation and the user just needs to specify the Kd, Ki, Kp, setpoint values, the variable that is being controlled and the variable that will be adjusted by the PID.

Arduino (Banzi and Shiloh 2014) is an open-source electronics platform used to control electronic circuits by sending data and instructions to the microcontroller using Arduino IDE (Integrated Development Environment). Arduino boards can read inputs and turn it into an output and is widely used to control instruments, including projects involving temperature control with the use of PID libraries (Asraf et al. 2017; Mathew et al. 2015; Wang

and Chi 2016; Zermani et al. 2014). Arduino consists of both a programmable circuit board (microcontroller) and a piece of software that runs on a computer used to write and upload computer codes, usually C/C + + , into the physical board (D'Ausilio 2012). There are just a few low-cost portable PCR devices that use conventional PCR vials described in the literature while professional instruments can cost up to US$ 140,000. Table 1 shows a compilation of "low-cost" portable devices that uses conventional PCR tubes described in peer-reviewed manuscripts and over the internet. It's important to emphasize that the estimated price of a lab-made device in general does not include engineering costs. Usually, a factor of 1:10 between cost and price applies. For example, for a cost of goods of US$ 50, a device would probably be retailed for at least US$ 500.

In this article we show the construction of a low-cost PCR device (US$ ~ 50 in parts) that uses printed circuit boards (PCBs) as heating elements used to heat PCR tubes. The instrument was successfully tested through the amplification of DNA and RNA templates of Syphilis (bacteria) and Zika virus, respectively.

2 Material and methods

2.1 PCB-heater fabrication

Two double side PCB boards (FR4 of 35 μm of copper) were manufactured using the milling and drilling PCB machine LPKF S103 (LPKF Laser & Electronics, Germany). The electronic designs were made using the software's Eagle (version 9.2.2) and AutoCAD (student version), both from Autodesk (Autodesk, Inc. USA). The copper resistor designed on the bottom PCB board has a total resistance of 0.9 Ω designed around a 3.5 mm diameter hole that runs through both sides of the board. The top PCB board contains a hole of 8.5 mm diameter containing a small copper tube of 7 mms fitting on the hole and soldered on the superior copper layer. In this board the resistor was positioned in the bottom side only, and contains a resistance of 1.1 Ω. In both PCB boards there are surface mounted devices (SMD) NTC (negative temperature coefficient) thermistors of 10 kΩ used as temperature sensors soldered near to the resistor. In order to prevent oxidation of the copper resistors a ~ 1 mm layer of an epoxy resin (Advanced Vacuum HT 1564—Hi-tech composites) was deposited on top and fully cured at 80 ºC for 4 h. During the curing process the polymer that initially was transparent became slightly orange. This resin can withstand temperatures up to 150 ºC. The manufacturing procedure of the bottom heater can be seen in Fig. 1.

Table 1 Comparison of 'low-cost' portable instruments found in the literature and over the internet

Portable PCR Device	Heated Lid	Heating Ramp Rate	Cooling Ramp Rate	Standard tubes	Detection	Battery Power	PID control	Samples	Price ($)
Low-Cost PCR	Yes	2 ℃/s	2 ℃/s	Yes	No	Yes	Yes	1	~50
miniPCR™ mini8 thermal cycler (Min-iPCR 2019)	Yes	2.4 ℃/s	1.7 ℃/s	Yes	No	Yes	Yes	8	~650
Open PCR (OpenPCR 2016)	Yes	1 ℃/s	1 ℃/s	Yes	No	No	Yes	16	~499
miniPCR™ mini16 thermal cycler (MiniPCR 2021)	Yes	2.4 ℃/s	1.7 ℃/s	Yes	No	Yes	Yes	16	~840
TTC (Wong et al. 2015)	No	5 ℃/s	6 ℃/s	Yes	No	Yes	No	~4	~130
Arduino PCR (Stacey 2019)	No	-	-	Yes	No	No	Yes	2	~85
Palm PCR (Ahram Biosystems 2021)	No	-	-	No	No	Yes	Yes	12	~5 K

2.2 PCR block device

The PCR block was made using 4 screws (3/16 " diameter and 2" high) with the heads positioned at the base. A computer fan of (12 V, 0.04 amps) 4×4 cm and the two PCB boards were inserted on the screws as visualized in Fig. 2. The plastic PCR vial is positioned within the copper tube (that holds and heats the PCR body) and the vial base containing the reaction fits within the bore of the bottom PCB plate where the temperature cycles occur.

2.3 Electronics

The main board contains the Arduino Nano microcontroller, three IRF MOSFETs 3415 used to turn ON and OFF the

PCB-made resistors (1.1 and 0.9 Ω), and the fan; three resistors of 1 MΩ, two of 220 Ω, one simple LED, one RGB LED and two capacitors (ceramic of 100 nF, electrolytic of 33 μF). The schematic diagram of the instrument is shown in Fig. 3. Three voltage dividers were made using three 10 kΩ resistors and the SMD NTC thermistors, two of them to measure the temperature in both boards and one to calibrate the system. The instrument was powered by an AC/DC adapter (input 100-240 V—50/60 Hz—output 12 V, 5 A (FY, W-T5000 – Hechoen-China).

2.4 Software

It was created a visual interface to interact with the device, where it is possible to control the main parameters,

Fig. 1 PCB-heater fabrication of the bottom heater using a CNC machine. **a** PCB board; **b** Copper resistor manufacture using a CNC machine; **c** Welding of electronic components; **d** Epoxy coating; **e** Epoxy curing and **f** Final PCB-heater device
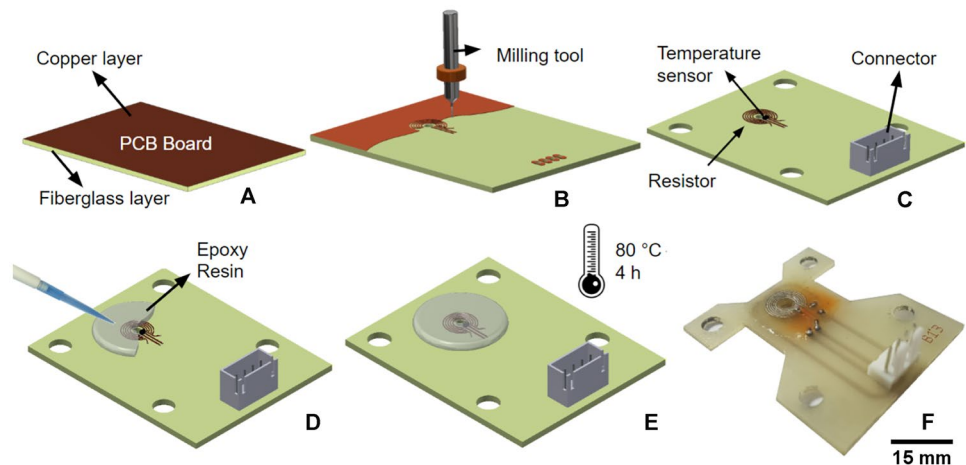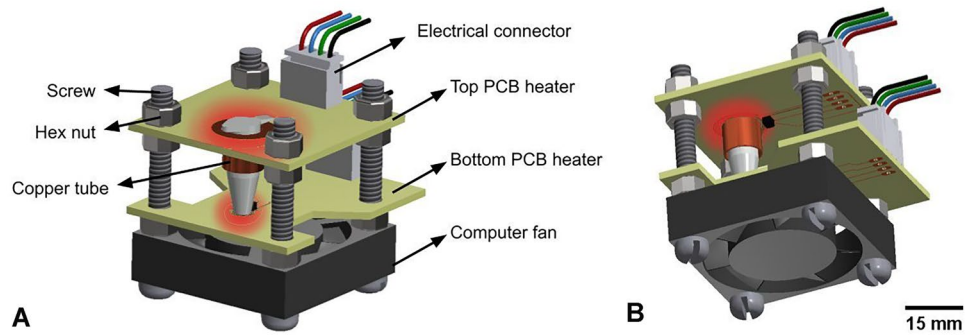
Fig. 2 Schematic diagram of the PCR Block. Top **a** and bottom **b** views. The bottom PCB heater board is the same represented in Fig. 1

understand the behavior of the sample via a chart, calibrate the device if necessary and obtain reports. The objective is to make a friendly interface where anyone can understand and set the desirable values in each cycle and save the obtained data on a. *mdb* file for further analysis. This software was built using the Demo version of Elipse Software (version 4.8.355) that contains the Elipse Studio to edit the interface, Elipse Viewer to execute the interface as well as the real time monitoring, and the Elipse Server, to store all processed data. The real time information is obtained through serial communication with Arduino using modbus protocol, and it uses special libraries that allows both Arduino and Elipse to communicate. It was implemented 5 separated PID algorithms on this prototype due to the different steps involved at the PCR process and the precision needed.

2.5 Samples, DNA or RNA extraction (RT)-PCR and gel electrophoresis

DNA of *T. pallidum* bacteria was obtained from Vircell AmpliRun[R] positive control. Zika virus RNA was extracted from positive control from ZDC Biomol Diagnostic kit (IBMP-PR-Brazil) using QIAamp Viral Mini Kit (QIAGEN). PCR reactions were performed in 20 μL final volume. *T. pallidum* DNA and Zika virus RNA samples were amplified using Universal PCR Master Mix (IBMP). The DNA target reaction was performed using the following cycle condition: 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The RNA target reaction was performed with the cycle condition: 51 °C for 30 min, 95 °C for 15 min, 40 cycles of 95 °C
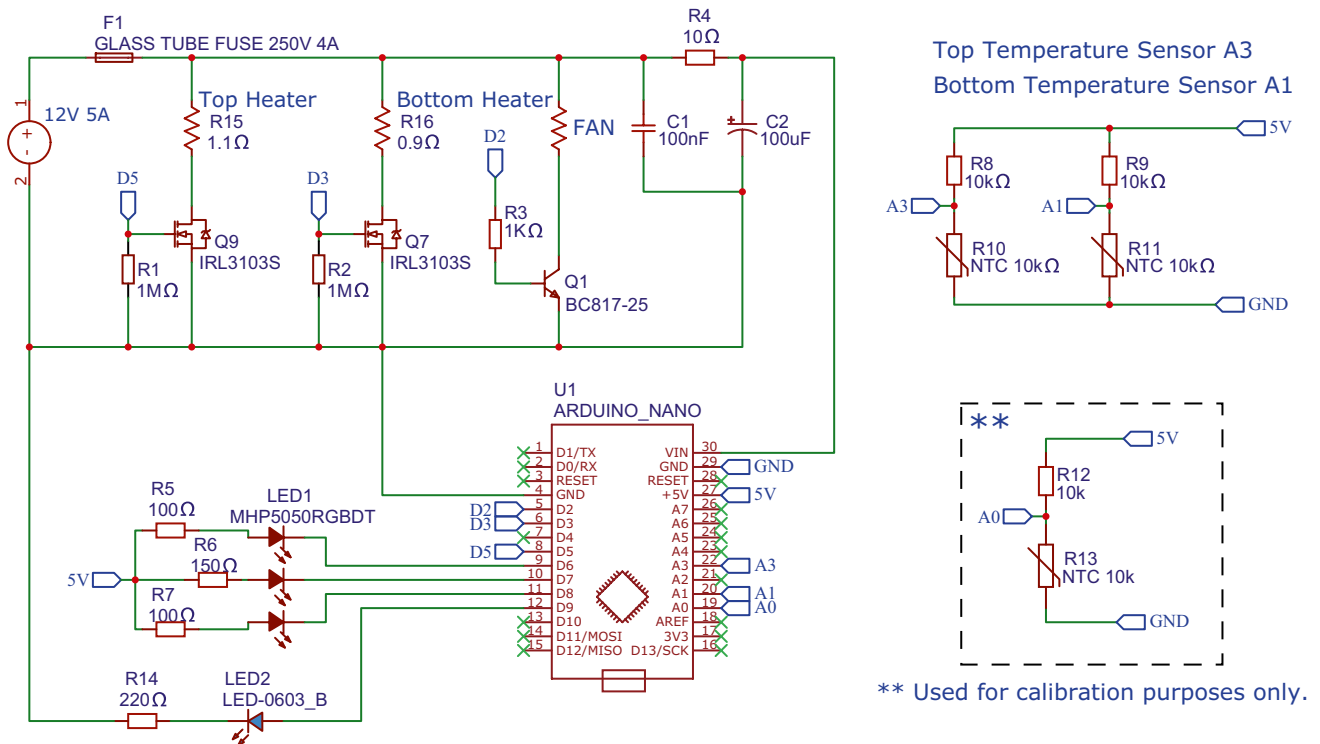

Fig. 3 Electronic Schematic Diagram of the PCR instrument

for 15 s and 60 ℃ for 1 min. The amplified product was loaded in an agarose gel 2% containing 0.4 µg/mL ethidium bromide, and the electrophoresis was conducted in TBE buffer at 100 V for 30 min. After the electrophoresis the DNA bands were visualized by UV transillumination.

3 Results and discussion

3.1 PCB heater manufacture

Conventional PCR devices heat whole 200 µL vials when generally only 10% of the volume is used. As, in general, only 20 µL needs to be heated, we have decided to create a low-cost active heater using the copper present on conventional PCB boards. During the manufacture we realized that when the copper channels were built too thin, they were very unstable, burning easily. If too thick, it does not act as a heater, but as a short circuit. The best compromise was achieved using 215,9 µm width. The resistor was designed around a 3.5 mm hole where the conic vial is seated, heating just the area where the liquid is. Near the hole center there is an SMD NTC thermistor, to measure the board temperature, that is correlated with the temperature inside the vial using a calibration curve made with an external sensor. In order to increase the heat and cooling efficiency at the bottom board we minimized the PCB area around the resistor to minimize heat dissipation. As the bottom board, the top board also has a copper spiral resistor, but its hole is 8,5 mm in diameter containing a small copper tube welded in the hole (see Fig. 1), where the temperature was maintained at 110 °C, avoiding condensation of sample on the vial body and lid. The external holes in both boards are spaced 32 mm apart, in order to match the holes already present in the fan, so they can be connected by screws. Using this simple configuration with the fan positioned under the bottom plate and two PCB boards tightly secured with screws and nuts, we developed a simple PCR block that can be easily reproduced (see Fig. 1). The highest current drawn in our system was 1.8 A.

3.2 Calibration

The instrument calibration is necessary to ensure the accuracy of the temperature inside the PCR tube. The first step was to create a calibration curve comparing the temperature on the board and the temperature inside the liquid. The temperature on the board was measured with the SMD NTC thermistor soldered on it, and the liquid temperature was measured using another SMD NTC thermistor connected to the main board (see Fig. 3). This external sensor was inserted inside of a 200 µL PCR Eppendorf tube containing 20 µL of water and sealed in order to simulate a real scenario. A curve and its respective equation are created in this process and although the temperature response is quite linear, there are some points at high temperatures that are not totally covered by a linear regression as expected, as can be seen in Fig. 4. These variations can be adjusted through the code to make corrections based on any difference that can be noticed throughout the calibration.

In order to understand the influence of the heating of both boards in the liquid temperature, we performed experiments turning ON and OFF each board separately. First, with just the top board at 110 ℃ and the bottom board turned OFF, the water temperature increased from 25 ℃ to 54 ℃. This shows that heat is transferred from the top board to the liquid, as expected, and that the top board temperature also needs to be controlled in order to avoid interferences during the cycles. This temperature is also sensed on the bottom board temperature sensor. With the top board turned OFF the bottom board needs to heat to around 117 ℃ to achieve 95 ℃ inside the liquid, that is not the best situation since the plastic vial can melt at very high temperatures. Also, the excessive temperature on the board could potentially damage it along the time. It's also verified that the top temperature sensor does not feel any significant change in temperature while heating the liquid to 95 ℃. These results show that both boards are contributing to the heating, and the resulting temperature depends on both temperatures that needs to be precisely controlled. These results are shown in Table 2.

3.3 PID control

All PCR cycles were realized using PID control settled by the Arduino program. This operation process was created using five PID controllers, each one responsible for one step of the process, as shown in Table 3.

As is shown in Table 3 there are five PIDs implemented on the software. PID 1 is responsible for maintaining the temperature of the activation step at 95 ℃; PID 2 is responsible for stabilizing the annealing and extension steps temperature at 60 ℃; PID 3 is responsible to hold the temperature at 95 ℃ in the denaturing step; PID 4 was created in order to increase the temperature rise rate to 95 ℃; PID 5 is responsible for the reverse transcription step.

The implementation of these PIDs were essential to the precise temperature control.

Figure 5 shows an example of a cycle. This graph is very similar to the ones observed in commercial instruments.

The PID parameters were chosen based on the system response via manual tuning, choosing first the proportional parameter, then the integral parameter and lastly the derivative parameter. At first, the I and D parameters were set to

Fig. 4 Linear regression of the NTC thermistor temperature soldered on the bottom board and the external NTC thermistor inserted on the vial (for calibration)
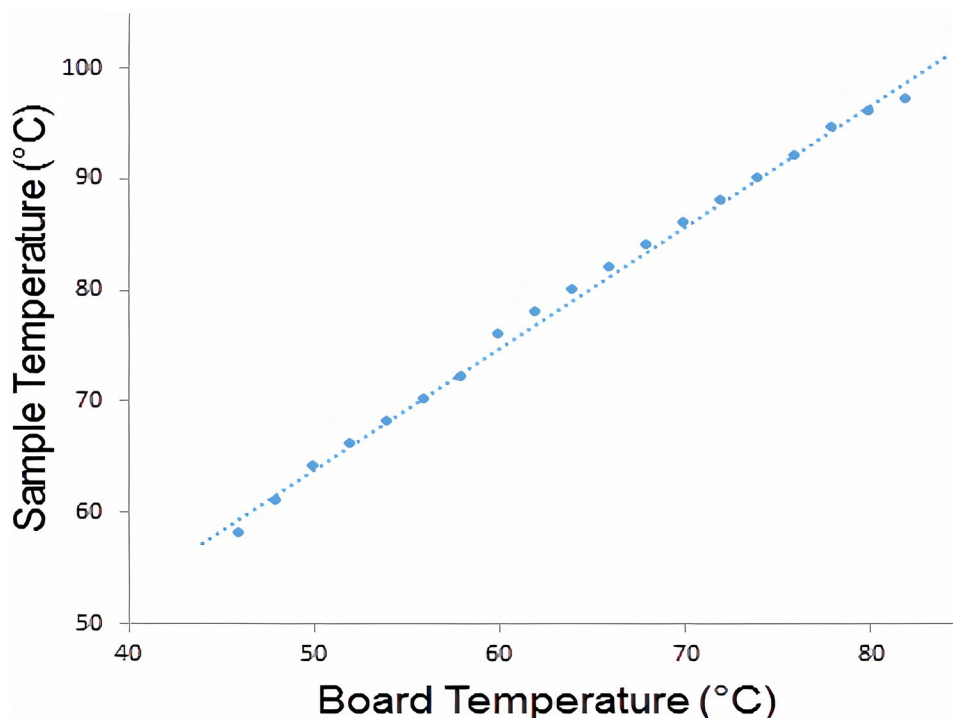
0 and the P parameter was chosen in order to get the output of the system close to a given setpoint. Once the P parameter was set, the I parameter was adjusted to correct any offset that the output could have in relation to the setpoint; and finally, the D parameter was tuned in order to increase overall stability in the output. The temperature precision in our instrument was ± 1 °C (20 to 95 °C), while commercial instruments are around ± 0.2 °C (20 to 72 °C) and ± 0.4 °C (temp higher than 72 °C). These differences in accuracy are totally expected since our electronics and mechanics is simpler in comparison with commercial instruments.

3.4 DNA analysis

To test the low-cost PCR equipment, three samples were amplified in our device and compared with the commercial ABI 7500 Real-Time PCR System (Thermo Fisher Scientific). Two positive controls and one negative control were

used. The rate of increase and decrease of temperature in the ABI 7500 is ~ 1.1 °C/s, while the low-cost PCR is ~ 2.0 °C/s, almost twice faster. There are many instrumental parameters that can explain these differences, therefore we are not saying that our device is better than the commercial instrument, they have completely different capabilities. However, it's evident that our instrument works as expected. Figure 6 shows the amplification of DNA and RNA fragments using both instruments. The sense and antisense primers of flaA gene (*T. pallidum*—M63142.1) are positioned at bases 316 to 333 and 376 to 359 respectively, generating the 61-bp PCR product. The Zika virus amplicon has 76-bp, the sense and antisense primers are localized at the bases 1086–1102 and 1139–1162 in the virus genome. For analyzing multiple primer sequences, we used the Multiple Primer Analyzer Tool (ThermoFisher Scientific) for primer dimer detection and these oligonucleotides do not form self-dimers and cross primer dimers.

Table 2 Temperature behavior inside of a sealed 200 μL PCR Eppendorf tube containing 20 μL of water

Heater	Top Board (T°C)	Bottom Board (T°C)	Water final (T°C)
Top (ON) Bottom (OFF)	110 ± 1	54 ± 1	59 ± 1
Top (OFF) Bottom (ON)	26 ± 1	107 ± 2	95 ± 1
Top (ON) Bottom (ON)	110 ± 1	95 ± 1	95 ± 1

Table 3 Parameters used for PID calibration on the instrument firmware. PID values 1 to 5 are related to: Activation step; Annealing step; Denaturing step; Rise signal and Reverse Transcription

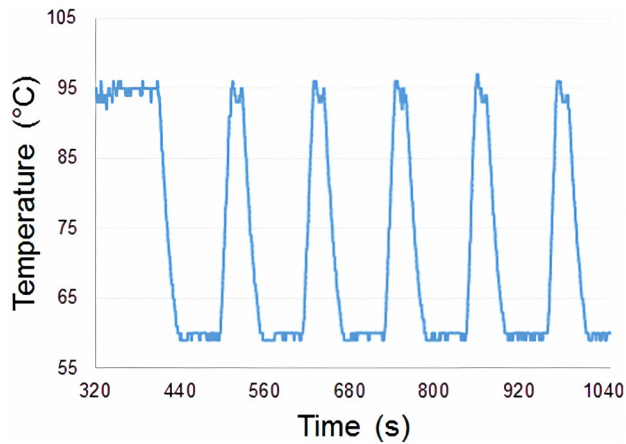| Algorithm	Target Temp. (°C)	Temp. Range (°C)	Kp	Ki	Kd
PID 1	95	93–96	1.75	1.5	12.0
PID 2	60	58–62	15.0	2.5	6.25
PID 3	95	93–97	11	0.32	15.0
PID 4	95	93–98	1.75	1.5	1.25
PID 5	51	48–52	15.0	1.0	8.0

Fig. 5 Typical amplification graphic obtained in our low-cost PCR prototype using a PID control. Parameters: cycles of 95 °C for 15 s and 60 °C for 1 min
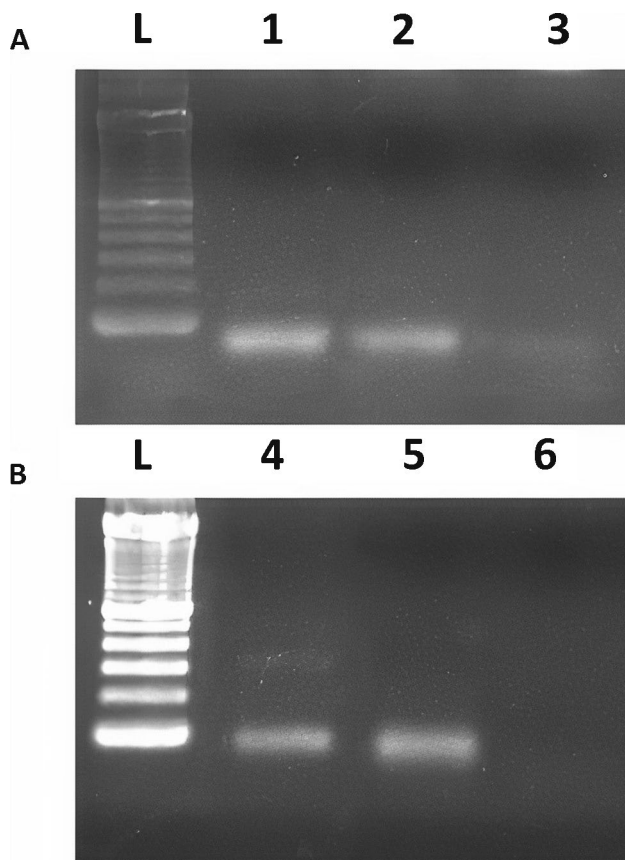

Fig. 6 Gel electrophoresis analysis. (A)—PCR reaction—*T. pallidum (Syphilis)* DNA sample; L. 100 bp DNA Ladder (Invitrogen™); 1. positive control DNA (ABI 7500); 2. DNA positive (low-cost PCR); 3. negative DNA control (low-cost PCR). (B)—RT-PCR—Zika virus RNA sample: 4. positive RNA (ABI 7500); 5. positive RNA (low-cost PCR); 6. negative RNA control (low-cost PCR). As can be seen both instruments amplified Syphilis DNA and Zika RNA targets as expected, showing similar performance

4 Conclusions

A low-cost PCR instrument was created using customized PCB boards as heating elements and a small computer fan to cool down the reaction. A PID control system was implemented combined with a temperature calibration system, in order to control precisely the temperature. The PID control was implemented using open-source Arduino libraries. During the PCR and RT-PCR reactions we used a variety of cycles in which we raised the temperature from ambient temperature to 95 °C. The final device was able to heat and cool down the reaction at rates of 2.0 °C/s. This value is comparable with the heating (1 to 2.4 °C/s) and cooling (1 to 1.7 °C/s) rates verified in the Minipcr (www.minipcr.com) and the Openpcr devices (openpcr.org), that are also considered ``low-cost`` instruments. We also checked that the heat and cooling rates observed in the low-cost PCR is also comparable with the heating (1 to 6 °C/s) and cooling (2 to 6 °C/s) rates observed in 15 high standard instruments found in the market. This shows that standardized versions of our board may be used in future to create cheap instruments, avoiding the use of aluminium blocks and peltier elements. The low-cost PCR showed similar results in comparison with the PCR ABI 7500, both in amplification plot and gel electrophoresis analysis. The low-cost PCR device is effective, portable and easy to handle, making it user-friendly. The instrument works with a 12 V external power supply and may be used in combination with a conventional battery. Although this instrument was designed to operate with just one sample, the same principles here applied can be used to run many samples in combination with microchip devices or the use of a bigger board with multiple vials. Costing nearly US$ ~ 50, the low-cost PCR device was tested successfully in the detection of Syphilis and Zika virus, showing that simple technology is a viable alternative for diagnostics in laboratories with low financial resources. We also show for the first time that customized PCB boards may be used in future as a heat element in PCR instruments, not only in Lab on a Chip devices. In order to provide the community with a fully open-source device, we also shared the firmware and software in the GitHub (https://github.com/BrunoDCamargo/low-cost-PCR-instrument) repository commonly used to host open-source projects.

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