A Portable 3D-printed Platform for Point-of-care Diagnosis of Clostridium difficile Infection and Malaria

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There is a critical need for better low-cost portable diagnostic platforms that can be used in both hospital and resource-limited settings. Here, we integrate 3D-printing technology with low-cost open source electronics to develop a portable diagnostic platform suitable for a wide variety of diagnostic and sensing assays. We demonstrate two different clinical applications in the diagnosis of *Clostridium difficile* infection and malaria. For *C. difficile* diagnosis, we used the portable diagnostic platform integrated with loop-mediated isothermal amplification (LAMP) to demonstrate robust and specific detection of DNA. The device was capable of identifying *C. difficile* with 100% accuracy using the ten most common *C. difficile* PCR ribotypes. The device was then adapted as a malaria diagnosis tool suitable for resource-limited settings. The malaria aptamer-tethered enzyme capture (APTEC) assay was integrated with the portable device through integration of 3D-printed components. The device detected malaria biomarker protein *P. falciparum* lactate dehydrogenase (*Pf*LDH) from simulated blood samples with a sensitivity in a similar clinical range to a laboratory-based spectrophotometer. By exploiting the broad customizability of 3D printed devices, this portable diagnostic platform could be applied to a wide range of other clinically relevant pathogens for rapid and accurate diagnosis within hospitals, clinics and resource-limited settings.

1. Introduction

There is a growing need for portable, low-cost diagnostic tools to allow urgent clinical decisions to be made more quickly, thus leading to better medical outcomes and lower healthcare costs.[1] Such tools would benefit hospitals and clinics which face increasing healthcare costs.[2] Moreover, such tools are critical to low-resource settings where access to rapid diagnostic tests has significantly improved public health.[3] In hospital settings, point-of-care (POC) tests have been used routinely for over 50 years.[4] Today, POC tests are used for diseases including hepatitis C, influenza A & B and HIV, [5] but many clinical decisions still take too long. For example, detection of the presence of antibiotic-resistant *Clostridium difficile* (*C. difficile*) relies on either the stool cytotoxicity assay (CTA) or toxigenic culture (TC), procedures which can take up to 72 hours to complete.[6,7] To aid decision making, clinical laboratories routinely screen patient samples using enzyme immunoassays (EIAs) to overcome the time constraints of TC.[8] However, EIAs can lack sensitivity and specificity[9] and it is therefore necessary to use two to three methods per patient to diagnose *C. difficile.*[10] Several clinical studies have demonstrated that quantitative real-time polymerase chain reaction (qPCR) could replace the current diagnostic assay.[11-13] However, devices to perform qPCR are still costly and not generally available at the point-of-care.

Outside hospital settings, better POC testing is needed for diseases that are endemic to resource-limited settings. Malaria is a classic example. Whilst malaria mortality rates are falling, the disease is still estimated to cause over 400,000 deaths annually.[14] The highest levels of mortality are found in localities that lack access to clinical facilities which provide the gold standard in malaria diagnosis, thick film microscopy.[15] This diagnostic challenge has spurred the development of malaria POC tests. 108 million POC diagnostic tests were distributed in 2012 for the diagnosis of malaria and have proven indispensable for effective patient treatment.[16,17] Thermosensitive antibodies are the active biomarker capture component in these POC tests. However, regions that lack cold chain supply see variability in test performance and reliability due to the degradation of the antibody components.[18, 19] Thermostable nucleic acid aptamer-based technologies have promise for malaria diagnosis.[20-23]

Assays have been recently developed for both antibiotic resistant bacteria and malaria that need new designs of device to facilitate clinical adoption. Assays to detect *C. difficile* using loop-mediated isothermal amplification (LAMP) have been demonstrated which are easy-to-use, rapid, robust, and affordable.[24] LAMP employs an isothermal DNA polymerase for amplification of target DNA and therefore does not require an expensive thermocycler.[25,26] LAMP produces magnesium pyrophosphate as a by-product during amplification leading to turbidity. The turbidity is proportional to the amount of DNA amplified and thus can quantify pathogenic DNA. For malaria, a robust assay developed to the requirements of endemic regions is the aptamer tethered enzyme capture (APTEC) assay.[22] APTEC uses a specific thermostable single-stranded DNA aptamer to capture malaria biomarker *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH). The biomarker can be identified by a colorimetric reaction, in which the captured *Pf*LDH catalyzes the production of a purple diformazan dye that can be easily quantified. However, for both of these scenarios, robust, inexpensive and

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sensitive point of care devices are needed to facilitate widespread clinical adoption.

Broadly speaking, POC diagnostic tools split into two categories: single-use hand-held tests/devices and devices requiring portable bench-top detection systems. While the former is widely used (e.g. home pregnancy test), the latter allows more sensitive and rapid detection, which minimizes the risk of false positive reporting [27]. However, the hardware platforms, are often still too costly, especially in resource limited settings.[28] Open-source hardware and manufacturing systems have led to innovative products at lower cost for the end user.[29,30] Open-source hardware allows for rapid product development and secure platforms that are simple to repair and maintain.[29] Although 3D printed materials do not yet have the resolution of PDMS microfluidics generated by soft lithography,[31] there are comparable examples of 3D printed microfluidics,[32,33] Furthermore, 3D printing can use a wide range of materials,[34] is less labour intensive,[35] and even low-cost commercial 3D printers are capable of facile replication of a range of customisable and scalable device components.[36] Advances in 3D printing technology have allowed the rapid prototyping of tools which simplify complex assay procedures. By combining 3D printing with open source hardware and novel biomedical diagnostic assays, we have prototyped devices that can benefit hospitals and resource limited clinics.

Herein, we present a portable electronic sensing device using an open-source Arduino microcontroller and 3D-printed reactionware and demonstrate its versatility by applying the device in two diagnostic assays. We demonstrate reliable detection of *C. difficile* isolated from patient samples by LAMP in the portable device capable of heating, measuring turbidity and producing a conclusive positive/negative result, see Fig. 1. Furthermore, we demonstrate how the device can be used to facilitate an aptamer-mediated colorimetric malaria POC test. The portable electronic sensing device has the potential to be applied in a wide range of diagnostic and sensing applications.
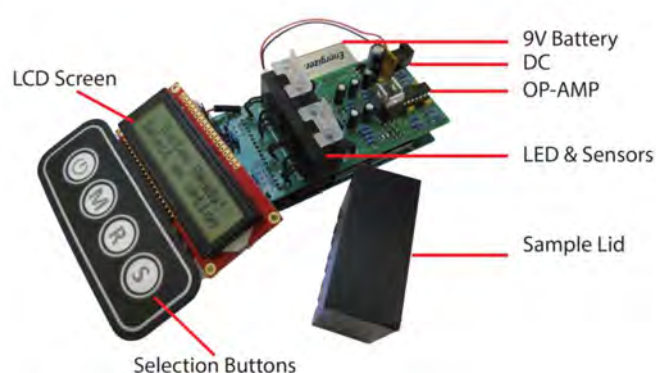

Figure 1. Portable 3D printed detection device. The 3D printed device is capable of heating to 63 °C and can measure the turbidity of the sample. The device is controlled using an Arduino and a bespoke PCB to control the heating and sample measurements. The on-board selection keypad can directly control the LAMP diagnostic device as well as a 9V battery that also powers it. Results are displayed on the integrated LCD screen which contributes to the portability of the device. The keypad buttons: **M**) Measure Absorbance; **R**) Run LAMP; **S**) System Initialization (heating).

2. Material and methods

Materials

Bst 2.0 DNA Polymerase for LAMP was purchased from New England Biolabs (UK). *illumi*gene® *C. difficile* molecular assay kit was purchased from Meridian Biosciences Inc. 2008s- biotin DNA aptamer was purchased from Integrated DNA Technology Inc. (Singapore). Dynabeads MyOne Streptavidin T1 beads were purchased from ThermoFisher Scientific (U.S.A.), Bovine serum albumin (BSA) from Affymetrix, and ultrapure agarose from ThermoFisher Scientific. VeroBlack 3D printing ink was purchased from SYS Systems (UK). All other chemicals were purchased from Sigma-Aldrich. Electronic parts for detection devices were purchased from Farnell (UK).

C. difficile detection by LAMP

All procedures were followed as laid out by the manufacturer protocol. 25 µL of *C. difficile* DNA samples were added into the provided reaction tube and preheated at 95 °C for 10 minutes. Formalin-treated *S. aureus* in phosphate buffer saline and 0.09% sodium azide was used as a negative control sample. For experiments with whole fixed bacteria, they were added to the control tube and treated as described above. Internal controls (positive / negative) were allowed to reach room temperature before being vortexed for 10 seconds and 50 µL of the respective controls were added into the provided reaction tube. All the reaction tubes were mixed by vortex for 10 seconds and 50 µL of the samples were added to both the control and test chambers of a single test device. The test device was placed into the 3D-printed thermo-chamber controlled by the bespoke PCB. The 3D thermo-chamber was heated to 63 °C and the absorbance was measured at the start of the reaction by using a 650 nm LED and a photodiode. The device was programed to measure the absorbance to detect the turbidity every 10 minutes. After 60 minutes, the final absorbance measurement was taken, and the sample was detected as either a positive or negative result. Criteria for positive or negative decision was the same as the assay using the kit and illumipro-10 commercial machine. The patient samples were measured blind and results reported and confirmed to Dr Douce.

Fabrication of the detection devices

3D-printed parts used on the device were fabricated using an Objet500 Connex™ 3D printer (Stratasys UK) using VeroBlack material, which provides opaque black printed parts for sensitive optical measurements. Custom printed circuit boards (PCB) that connect to Arduino™ MEGA ATmegaA2560 were designed using EAGLE PCB design software (Autodesk), and produced by Eurocircuits (Germany). Schematics for the 3D-printed parts are found in Figures S4-7. 3D parts used to enhance the APTEC assay were fabricated on an Objet500 Connex™ 3D printer or Freeform Pico printer in Plasclear Resin (Asiga USA). Schematics for these 3D-printed parts are found in Figures S19-S21.

Thermal measurement and control of portable device

Temperature control of the portable device was achieved by resistive heating composed of standard carbon film 6 Ω 0.25 Watt power resistors. The resistors are placed in between the PCR tube chambers equally spaced from each tube to ensure uniform heating to the lower parts of the PCR tubes. The temperature was controlled using a high power MOSFET driven with a pulse width modulated (PWM)

signal from the main control unit (Arduino). The PWM duty cycle was modified every 500 ms using a proportional controller algorithm. The temperature control algorithm relies on thermocouples, explained below, as a feedback system to record the current temperature and assign the PWM signal ratio depending on the error between the measured and the desired temperatures. Thermal sensing was performed using 100 Ω thermocouples placed directly under the chambers containing the PCR tubes. The output from each thermal sensor was fed into a second order resistor capacitor (RC) low-pass-filter with a cutoff frequency of 5 Hz, implemented to eliminate any coupled thermal noise from the neighboring heating elements. For accurate temperature measurement, the thermal readout was processed with a high resolution (10-bit) analogue to digital converter (ADC) available on the microcontroller. To further improve the temperature readout accuracy, 100 measurements are taken and averaged. The corresponding readout is compared to a lookup table containing calibrated temperatures. To achieve reliable and accurate temperature measurements, proportional control was used as a form of temperature feedback. The basic principle of the control system relied on heat supply or removal to compensate for the variation in the ambient temperature. This was achieved by using electronic temperature controllers, N-channel MOSFET (Metal oxide semiconductor field effect transistor) was used to vary the supply current through the resistive heaters and therefore increase or decrease the temperature of resistive heater. The term proportional refers to a value varying relative to another value. The output of a proportional controller was relative to the difference between the controlled temperature and the set point that is defined by the process or by the end user. The controller will turn the resistive heater fully on when the temperature is far below the set point and will turn the heaters off when the temperature is above the desired point.

Real-time turbidity monitoring for LAMP reaction

The turbidity measurement was performed using a high intensity light emitting diode (LED) with a wavelength of $\lambda = 650$ nm (TLDR580, VISHAY) and a photodiode (PD) (SFH203P, OSRAM). The LED and the PD are positioned perpendicular to the PCR tube, such that the light beam is centered at the lower part of the PCR tube. The PD measured the absorbance by measuring the light intensity as it corresponds to a photocurrent value. To minimize error and reduce measurement complexity, the photocurrent was converted to voltage and then amplified and filtered using a 2nd Sallen-Key Butterworth active low-pass-filter with a cutoff frequency of 5 Hz and a gain of 10. The signal obtained was then fed into a 10-bit analog to digital converter in the microcontroller. To further ensure accurate reading and reduce the signal to noise ratio, the system recorded the PD outputs 100 times nd average the results. The system featured an LCD display, user buttons for command entry, and a serial communication link. Results and system indicators were reported both on the display and streamed via the serial interface to the user PC application. The system can be powered sufficiently either from either a 9V battery or an isolated 9V mains power supply. The total current consumption when the device is in idle mode is 80 mA and at maximum peak operation, 748 mA. The entire custom device (Fig. 2) was fabricated and assembled using components costing less than $100.
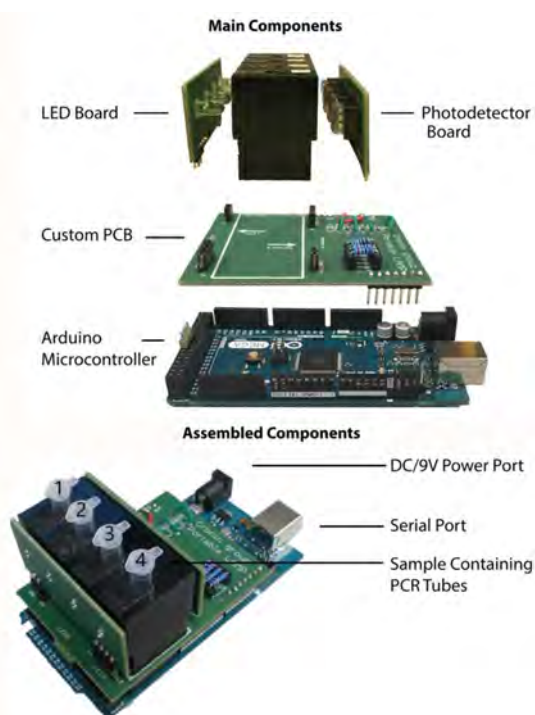

Figure 2. Modified portable 3D printed detection device for malaria diagnosis. The device has three key components: 3D printed housing which contains green LEDs (570nm) and sensors, a custom PCB, and an Arduino microcontroller.

Absorbance monitoring for APTEC assay

For detection of color changes by the APTEC reaction, a green LED with a wavelength of $\lambda = 570$ nm (HLMP-15, Broadcomm) and a photodiode (PD) with integrated operational amplifier (OPT101, Texas Instruments) were used. The op-amp integrated PD was employed for increased sensitivity. It also simplifies the device design and makes the system modularized by replacing an LED or PD array for colorimetric detection of other samples. Outputs of the PD sensors were recorded 100 times and averaged to filter out sensing noise. Sensor data was sent to a host computer via serial connection to store the data.

Absorbance monitoring for APTEC assay

Biotinylated 2008s single stranded DNA aptamers (5'-biotin- CTG GGC GGT AGA ACC ATA GTG ACC CAG CCG TCT AC -3') were non-covalently linked to a streptavidin-coated 96 well plate. Wells coated with the 2008s aptamer specifically capture malaria enzyme *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH). *Pf*LDH was recombinantly expressed in *Escherichia coli* strain BL21(DE3) pLysS and purified using affinity chromatography [21]. A known concentration of the recombinant *Pf*LDH protein was spiked into whole rat blood, and this surrogate whole blood solution was used as a substitute to *Plasmodium falciparum* infected human blood. An equal volume of blood substitute was added to lysis solution (2X PBS, 0.5% Triton-X100), which was incubated in an aptamer-coated well for 30 minutes. The incubation wells were then washed 3 times with wash buffer (PBS, 0.1% Tween-20). Development solution was freshly prepared and added to the incubation wells. With gentle rocking the plate was incubated at room temperature for 3 hours. The resulting solution was the scanned in a Thermo Scientific™ Varioskan™ microplate reader at 570nm or on the portable device.

3. Results and discussion

Device design and fabrication for LAMP device

A 3D-printed detection device was developed for potential use in hospitals as a portable and cost-effective tool for the diagnosis of *C. difficile*. We utilized a commercial *C. difficile* LAMP test (Meridian Biosciences) to detect *C. difficile* in clinical samples. Each clinical sample tested was always paired with a positive control sample to ensure the LAMP reaction was performed correctly. Two experimental conditions were required to run the LAMP assay: a constant temperature for isothermal DNA polymerase, and real-time measurement of solution turbidity to monitor DNA amplification. We designed a 3D-printed housing that incorporated resistive heating elements and pairs of LED and PD sensors (Fig. 1 and S1†). To limit the device to a palm-top size, a printed circuit board (PCB) that mounts on an Arduino microcontroller (hereafter, Arduino shield) was also designed to accommodate the 3D-printed housing (Fig. S2-4†). The device was powered with a 9V battery. The selection buttons and an LCD display were used to operate the device and display results. The device can also be connected to a personal computer via USB providing a 9V power supply and means to retrieve test results (Fig. 1).

An isothermal temperature was maintained using standard carbon film-based heating elements and thermistors. To enable accurate temperature measurements, we 3D-printed a custom housing which positions the thermistors below the PCR tubes. This close proximity allowed for accurate temperature measurements (Fig. S5† and S6†). A proportional–integral–derivative (PID) control was used to maintain the temperature at 63 °C, the optimal temperature for LAMP reaction. The turbidity of reaction mixture was measured by a 650 nm LED and PD sensor positioned on opposite sides of the sample PCR tube (Fig S2†). A small aperture between the LED and sensor in the black 3D-printed housing was designed (Fig S7-10†) which ensures detected light on the PD sensor was transmitted through the reaction mixture. The current generated by a PD sensor in response to incoming light was amplified with an operational amplifier (op-amp) on the PCB and converted to voltage, which was relayed back to the Arduino via an analog input. The decision criterion for a positive or negative result mirrors that of a commercial automated detection system (illumipro-10™, Meridian Bioscience Europe). The absorbance of a sample of interest at the start of the test (denoted as *i*) was first measured using the LED and sensor, and then repeated again after 60 minutes (denoted as *f*). If the positive control was valid (i.e. *f:i* ratio >0.82), the result for a sample of interest was determined as positive or negative. The results were either displayed on a PC screen via serial connection or on the built-in LCD screen. These processes are described in detail in Figures S11-15†.

Device performance using *C. difficile* LAMP detection kit

To confirm that the portable 3D-printed diagnostic device effectively utilizes the illummigene *C. difficile* LAMP kit, we performed a blind test of DNA isolated from patient samples. We tested samples containing the UK's 10 most common *C. difficile* PCR ribotypes, and used *E. coli and C. sordellii* as negative control samples. The device accurately detected all 10 samples and the negative *E. coli* control

within 60 minutes (Table 1). We were also able to accurately detect *C. sordellii* as negative, which is a common false positive in EIA tests. Furthermore, we tested fixed whole bacteria samples and accurately differentiated a *C. difficile* sample from controls (Table 1). These results demonstrated the possibility that our portable diagnostic device could function as an accurate POC device. It could potentially replace current EIA screenings as it can detect both toxin A/B positive *C. difficile*, while minimizing incidents of false positives caused by *C. sordellii*.

Device design and fabrication for malaria APTEC assay

We adapted the portable 3D-printed detection device for use in resource-limited regions (Fig. S16†) as it is capable of specifically detecting the colorimetric signal produced in the malaria APTEC assay (Fig S17). To increase specificity and sensitivity we modified the LAMP detection device by replacing light and sensory components, and simplifying the Arduino shield circuit. The device can be simply switched back to a LAMP detection device by swapping the Arduino shield and installing a firmware program to the Arduino board (see Supplementary Information).

Sample	Ribotype	Test	Control	Overall Result
1	001	+	+	Positive
2	002	+	+	Positive
3	005	+	+	Positive
4	014	+	+	Positive
5	015	+	+	Positive
6	020	+	+	Positive
7	023	+	+	Positive
8	027	+	+	Positive
9	078	+	+	Positive
10	106	+	+	Positive
11	*E. coli*	-	+	Negative
12*	*E. coli*	-	+	Negative
13*	027	+	+	Positive
14*	*C. sordellii*	-	+	Negative
Positive Kit Control	/	+	+	Positive
Negative Kit Control	/	-	+	Negative

Table 1. Device performance for *C. difficile* LAMP detection. Samples 1-10 and 13 are different ribotypes of *C. difficile*. Asterisks (*) indicate whole bacteria samples (10^6 CFU/mL). The results demonstrate that the device is able to amplify from both DNA and whole bacteria samples. Samples were blind tested and independently verified.

The APTEC assay generates a colorimetric solution which has the maximum absorption of light at 570nm.[22] We therefore used green LEDs (λ=570nm) to detect the colorimetric signal. We improved the signal-to-noise ratio by replacing the PD sensor and custom-designing an op-amp with IC chip containing a photodiode and transimpedance amplifier (OPT101, Texas Instruments). The IC chip also simplified the circuit design allowing modularization of device components. Therefore, LEDs can be simply exchanged in other colorimetric assays at different wavelengths. Two separate PCBs with four LEDs and PD sensors, respectively, were designed to dock onto

a modified Arduino shield (Fig. 2, Fig. S16[†]). Heating elements were removed from the PCB for simplicity as the APTEC assay can be performed at ambient temperatures. Finally, a 3D-printed housing was designed for the new LED and PD sensors and printed in an opaque black material for better sensitivity.

Malaria diagnostic device performance with simulated blood samples

Figure 3 illustrates the malaria diagnostic assay. To streamline the APTEC assay, we prototyped several improvements to make the assay both modular and capable of simultaneous testing of multiple samples. We designed and printed a four-pronged magnet transfer tool (Fig. 3A). This was designed to fit modular pre-prepared blocks as described in previous studies[37] and to fit 96 well plates, a readily available laboratory consumable. The design is shown in Fig. S18-20[†]. The 3D printed transfer instrument, composed of a biocompatible photopolymer,[31] provides a facile means of capturing magnetic beads and transferring them to different containers (Fig. 3B). This allows subsequent washing of beads and colorimetric assay (Fig. 3C) without impacting the assay specificity (Fig S21[†]).
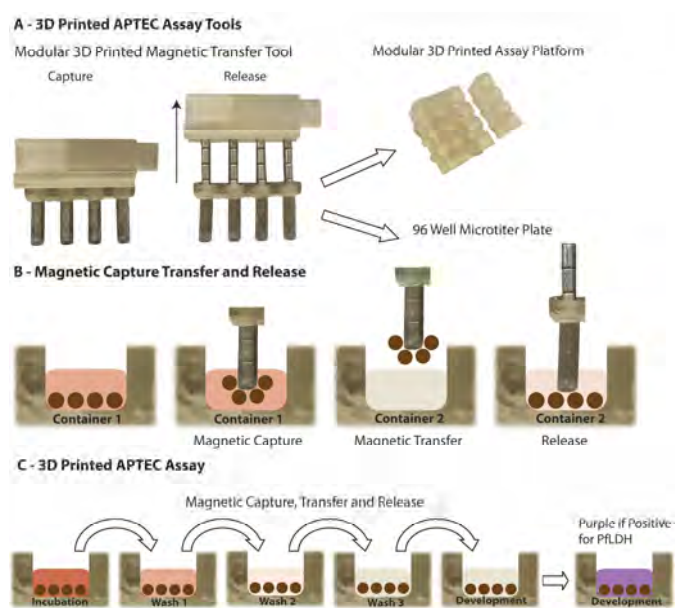

A - 3D Printed APTEC Assay Tools

B - Magnetic Capture Transfer and Release

C - 3D Printed APTEC Assay

Figure 3. **Design and use of the magnetic transfer tool in the malaria diagnostic APTEC assay**. A - The magnetic transfer tool is composed of a handle, cylindrical magnets and a cylindrical sheath. During capture, magnets are present within the cylindrical 3D printed sheath. During release, the magnets are lifted so that magnetic beads can fall into the container. The tool is sized for either a 96-well plate or modular 3D-printed assay blocks. B – The magnetic transfer tool is used to wash beads from non-specific blood components. Beads from Container 1 are magnetically attracted to the transfer tool. This tool can be lifted from the solution and transferred into a clean solution (Container 2). By removing the magnet from the sheath the beads are no longer attracted to the sheath and are suspended in the container solution. C - The first step in the APTEC assay is to incubate beads in a blood sample. These are then transferred by the magnetic transfer tool into three wash solutions. Finally, the beads are transferred into a development solution. If the blood sample contains the malaria enzyme *Plasmodium falciparum* the development solution will turn purple.

To carry out the assay, a 50 μL blood sample is added to the aptamer-modified micro-magnetic beads in the plate. There, the beads specifically capture *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH), the malaria biomarker enzyme. Next, the beads are washed several times to remove loosely bound non-specific blood components. Finally, the *Pf*LDH coated beads are transferred into a colourless development solution, where the *Pf*LDH reacts with the development solution to produce a colorimetric purple signal. Allowing the magnetic transfer tool to equilibrate in the development solution led to more consistent data (Fig S22A[†]). Three washes were sufficient to remove non-specific blood components (Fig S22B[†]). Using these 3D-printed tools we performed the APTEC assay and used the malaria diagnosis device to measure the signal intensity of the assay. We compared measurements using our malaria diagnosis device to measurements made on a laboratory-based 96-well colorimetric plate reader (Fig 4).
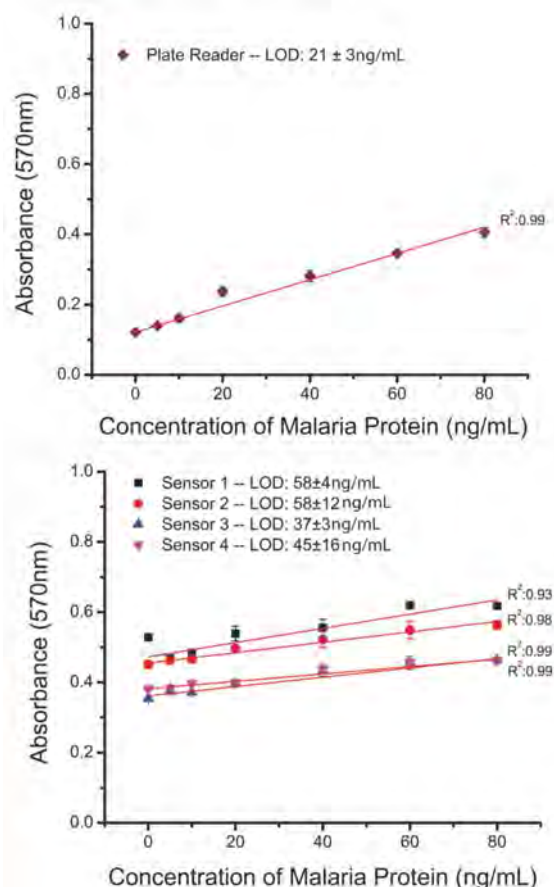

Figure 4. A comparison of the malaria APTEC assay between a laboratory-based plate reader (top) and the portable device (bottom). The enzyme concentration was assayed within the linear range of the APTEC assay. The portable version of the APTEC assay was used with the 3D-printed magnetic transfer tool. A – Detection of APTEC signal on a plate reader. B – Detection of APTEC signal on all four sensors. Limit of detection (LOD) was calculated as 3σ/m. σ was defined as the standard error of the regressed data and m was the gradient of the regressed data. Three independent regressions were performed for each LOD calculation.

The laboratory spectrophotometer showed a Limit of Detection (LOD) of 21±3 ng/mL for the malaria biomarker *Plasmodium falciparum* lactate dehydrogenase. The portable device showed a LOD in the range of 37-59 ng/mL across four sensors. Although this LOD was slightly less sensitive for the device relative to the

laboratory-based plate reader, the range was well within the clinical range for malaria diagnosis.[38]

Conclusions

We hypothesized that combining 3D-printed reactionware with open-source hardware could provide a new design for a point-of-care diagnostic device that could be used in a variety of settings. Here, we presented a new 3D-printed platform that can amplify DNA/RNA by LAMP for the implementation of a fully portable detection system capable of heating, measuring turbidity and producing a conclusive positive/negative result for clinical samples. Importantly this system can be produced and maintained at a fraction of the cost to design and manufacture in comparison to currently available equipment on the market (Table S1). Many well-equipped hospitals struggle with the burden of antibiotic-resistant pathogen *C. difficile* and require a technical staff and equipment for detection. This system could quickly and specifically identify *C. difficile*-containing clinical samples. It accurately distinguished *C. difficile* from *C. sordellii,* a common false positive pathogen. The specificity, portability and rapidity of this device coupled to the commercially available LAMP kit would complement current strategies to free hospitals of the *C. difficile* burden.

The device is amenable to customization for detection of multiple pathogens by multiple methods. We showed that this device could be customized to diagnose malaria when integrated with an aptamer-tethered enzyme capture (APTEC) assay. The modified device effectively served as an inexpensive spectrophotometer to detect the positive signal from the malaria APTEC assay without compromising measurement accuracy. We used 3D-printing to rapidly prototype new tools to be integrated into the APTEC assay. The sensitivity of the portable device was in a similar clinical range to a lab-based spectrophotometer at a far lower cost. Modularized device design combined with flexible 3D-printing design renders this device further customizable. 3D printing technologies render new features to POC systems that have often been difficult to achieve with conventional technologies. For example, the 3D printed magnetic transfer tool brings scalability to the system as samples can be easily processed in parallel. Rapid design-fabrication-test cycles allow development of multiple POC systems, which gives versatility to the POC systems, as we demonstrated above with *C. difficile* and malaria assays. This approach can be easily adapted to other biological assays to detect protein and oligonucleotide biomarkers for increased diagnostic accuracy.

The portability of this platform may open up new ways to perform POC assays at multiple locations. Both variants of these devices can be connected to a LED screen, a smartphone, computer or SD card and powered by a 9V direct current. Portable rechargeable batteries could power this device. These batteries could easily be charged in a hospital or in the field with existing technologies which require little infrastructure. The device provides a secure way to collect, transmit and profile presence of a disease, with clear benefits for telemedicine applications. A network of devices could be useful in tracking epidemics both at small-scale (such as across a hospital wing or small isolated community) or at large-scale (for epidemic monitoring). This 3D-printed platform can function as a multi-disease analytical device to be used anywhere from near-patient to highly specialized laboratories due to its versatility. The customizable nature of 3D-printing technology allows rapid prototyping or expansion of the device to include multiple test samples or to fully integrate extraction processes into the device. The study presented here acts as a proof-of-concept for a customizable, portable, low-cost device which can be adapted for a wide range of diagnostic and sensing applications.

Conflicts of interest

Author JAT declare inventorship of patent US 9000137B2 relating to the underlying aptamer used in this work.

Acknowledgements

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Supplementary Information

A Portable 3D-printed Platform for Point-of-care Diagnosis of *Clostridium difficile* Infection and Malaria

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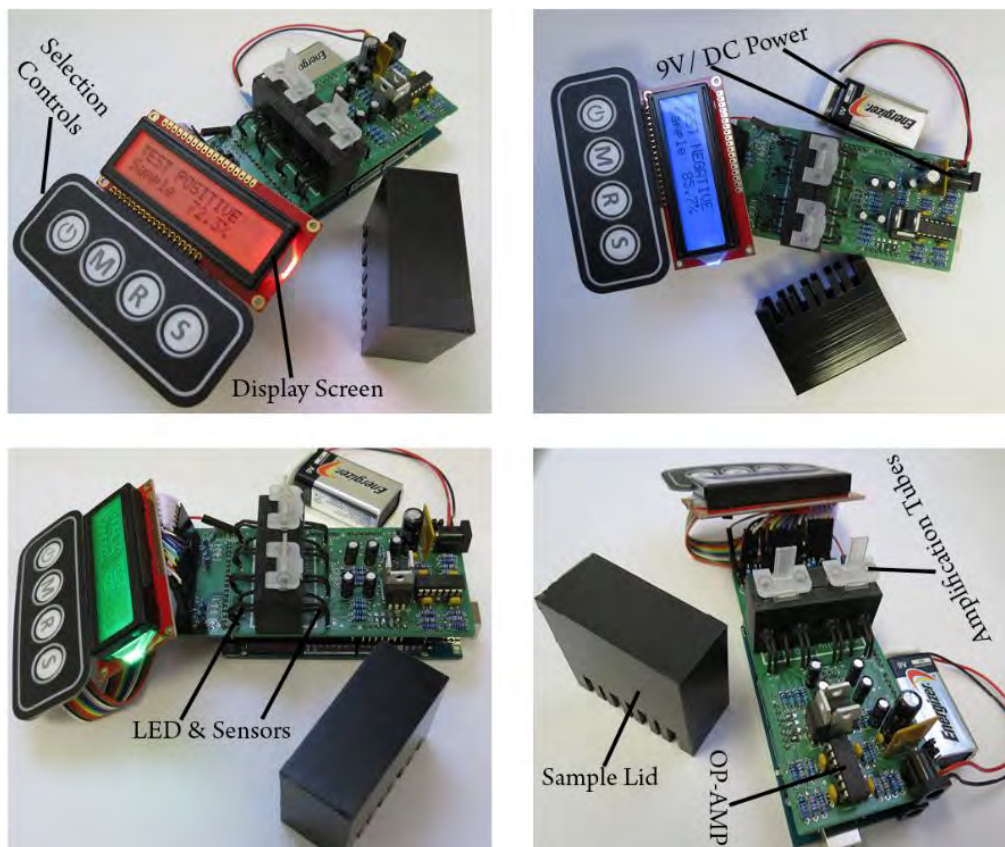**Contents:**

Overview of the device:

Figure S1 - Complete 3D printed device mounted on the customized PCB board that can be powered via a 9V battery for a period of 2 hours or a 9V DC power supply. The PCB Arduino shield can be connected via USB to a computer, however it can also operate standalone via integrated selection buttons and LCD display that allows the operator to power the device and results are displayed on screen.
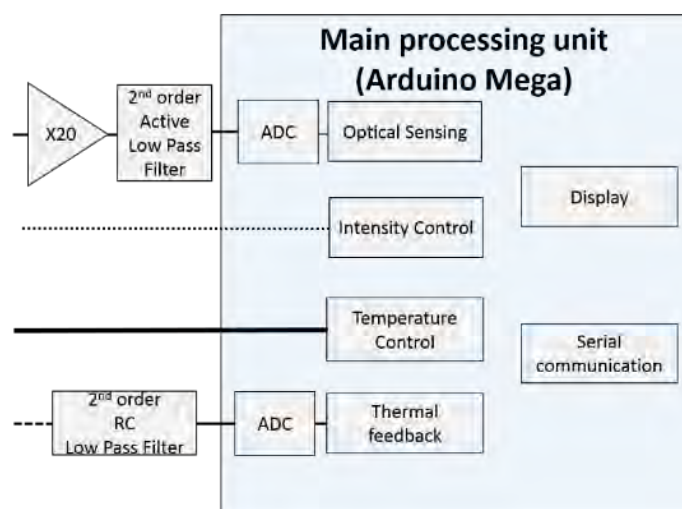
Figure S2 – System operational scheme. The system features an Arduino Mega, as a main processing unit on which our bespoke PCB shield attaches to. The PCB shield contains, a series of amplification circuits, followed by a second order low pass filter this is used for accurate optical turbidity measurements. In addition, it contains a proportional-integral-derivative controller (Proportional controller) for heat management.
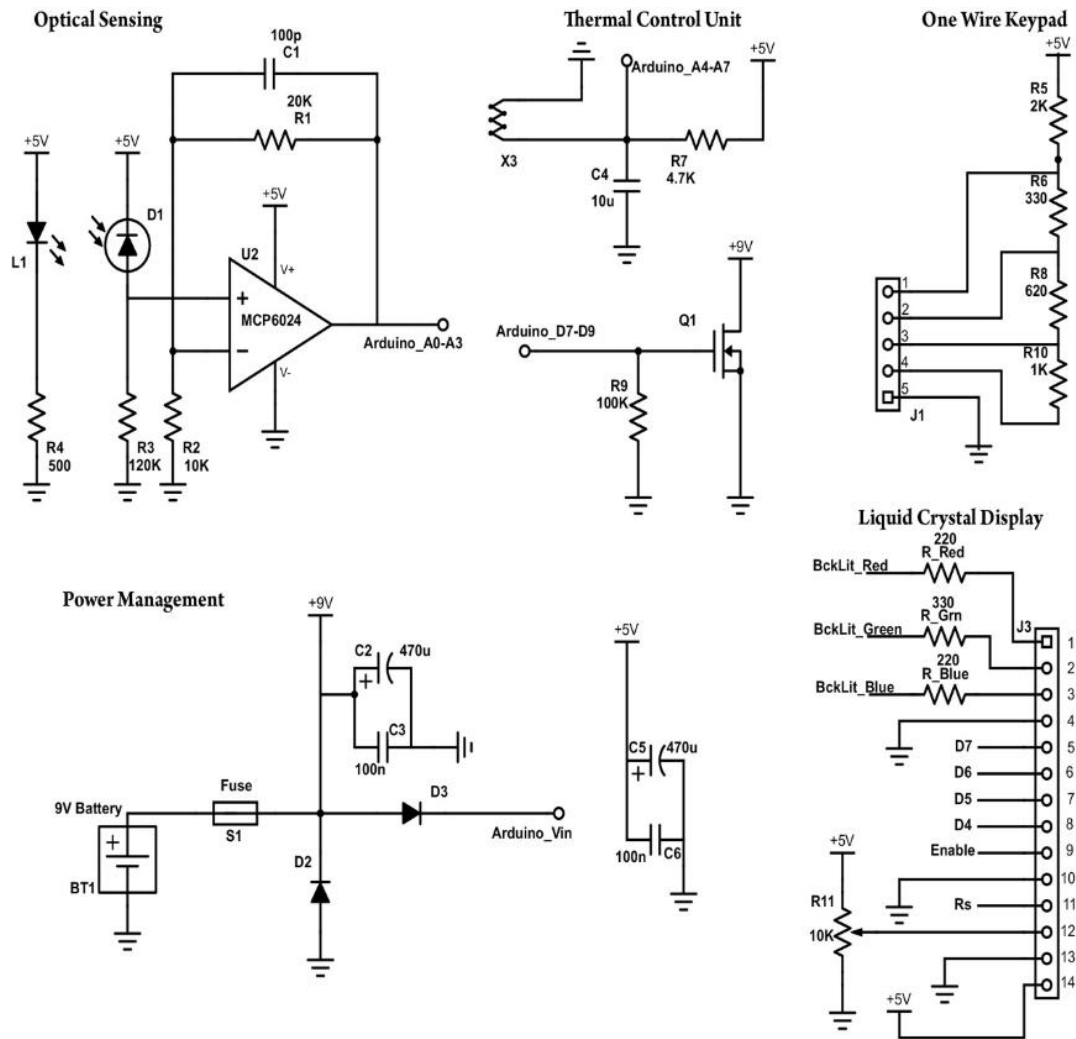
Figure S3 - PCB LAMP circuit schematic diagram illustrating the optical sensing circuit, power management unit, one wire keypad, LCD and thermal control unit including resistive heat drivers and thermocouples.
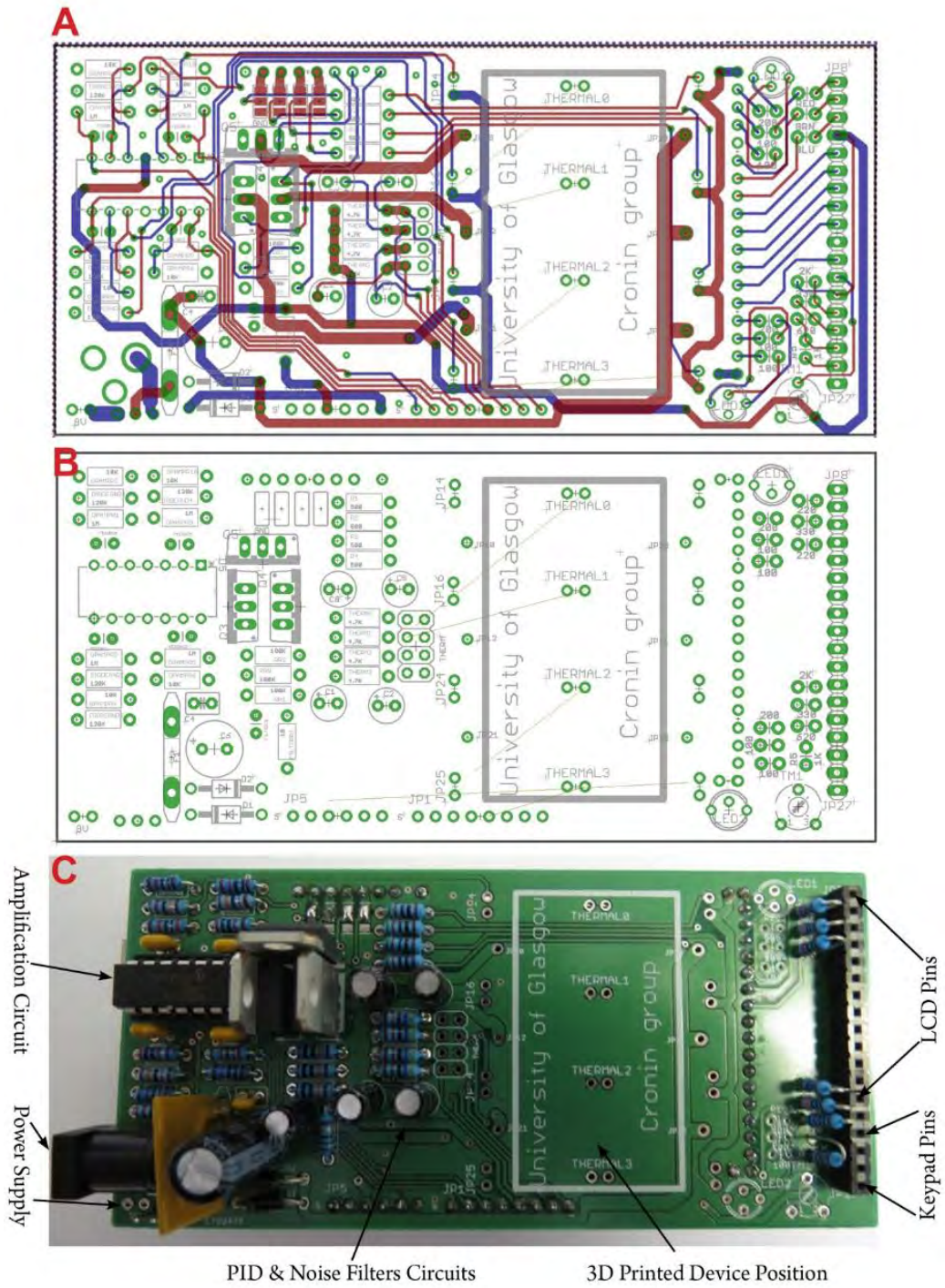
Figure S4 - **Complete LAMP system layout** (A) with list of components and placements (B) and fabricated PCB with component assembly (C).
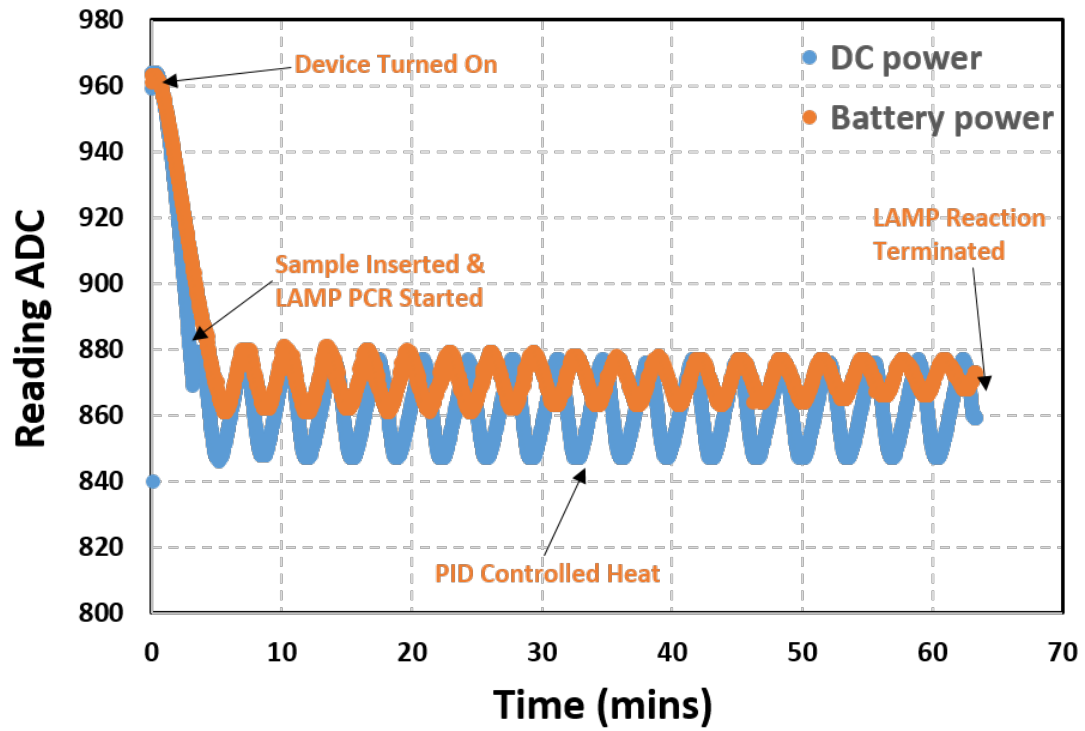
Figure S5 – **Illustration of portable LAMP PCR's proportional controller.** Heating is managed during the amplification step of the patient sample. The controller is essential to maintain a constant/correct temperature during the amplification stage.
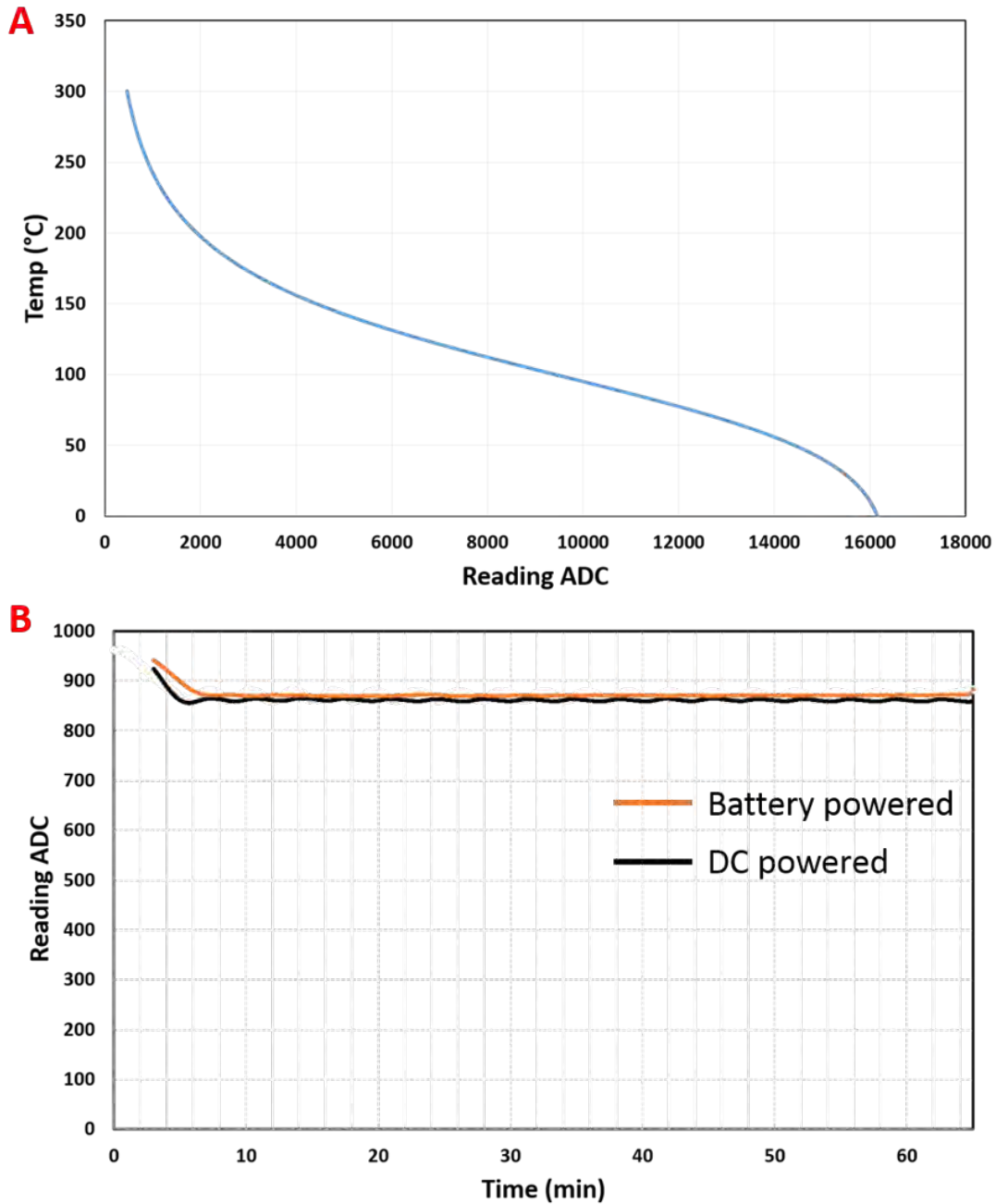
Figure S6 – 3D Printed Device heating profile A) thermocouple calibration curve extracted from marlin 3D printer. This calibration was used to set the temperature of the amplification device B) Temperature reading of 3D printed device, during LAMP PCR amplification, demonstrating a constant temperature throughout the amplification.
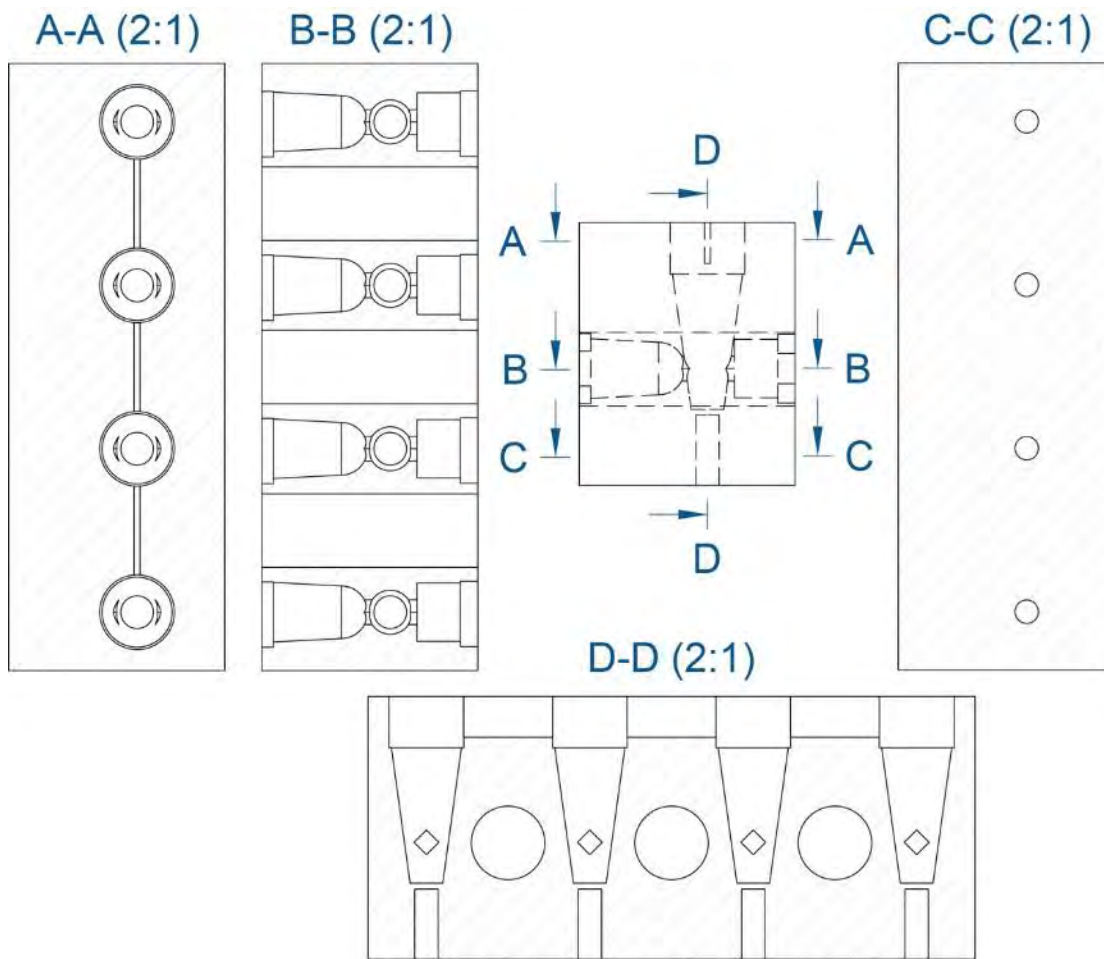
Figure S7– 3D printed device model and slices of the different sections of the device (slice A-D). This includes the tube holders, the LED and photodiode sensor holders as well as the cartridge heaters and thermistors. The lettering corresponds to the different slice outlined in the centre image.
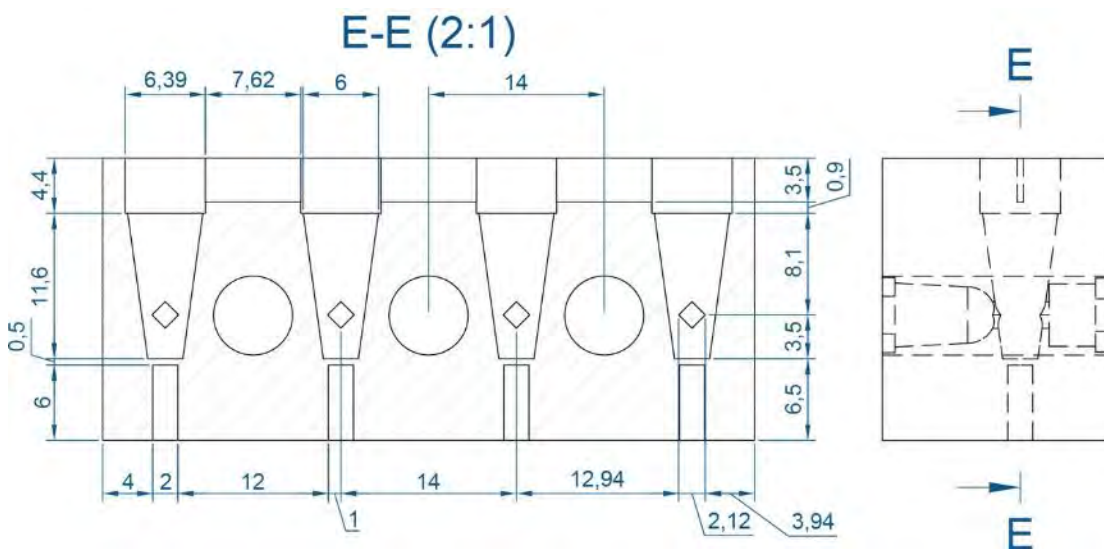
Figure S8 – 3D printed device model and a single slice of the device (slice E). The figure includes device dimensions and components that attach to the device. Millimetre measurements.
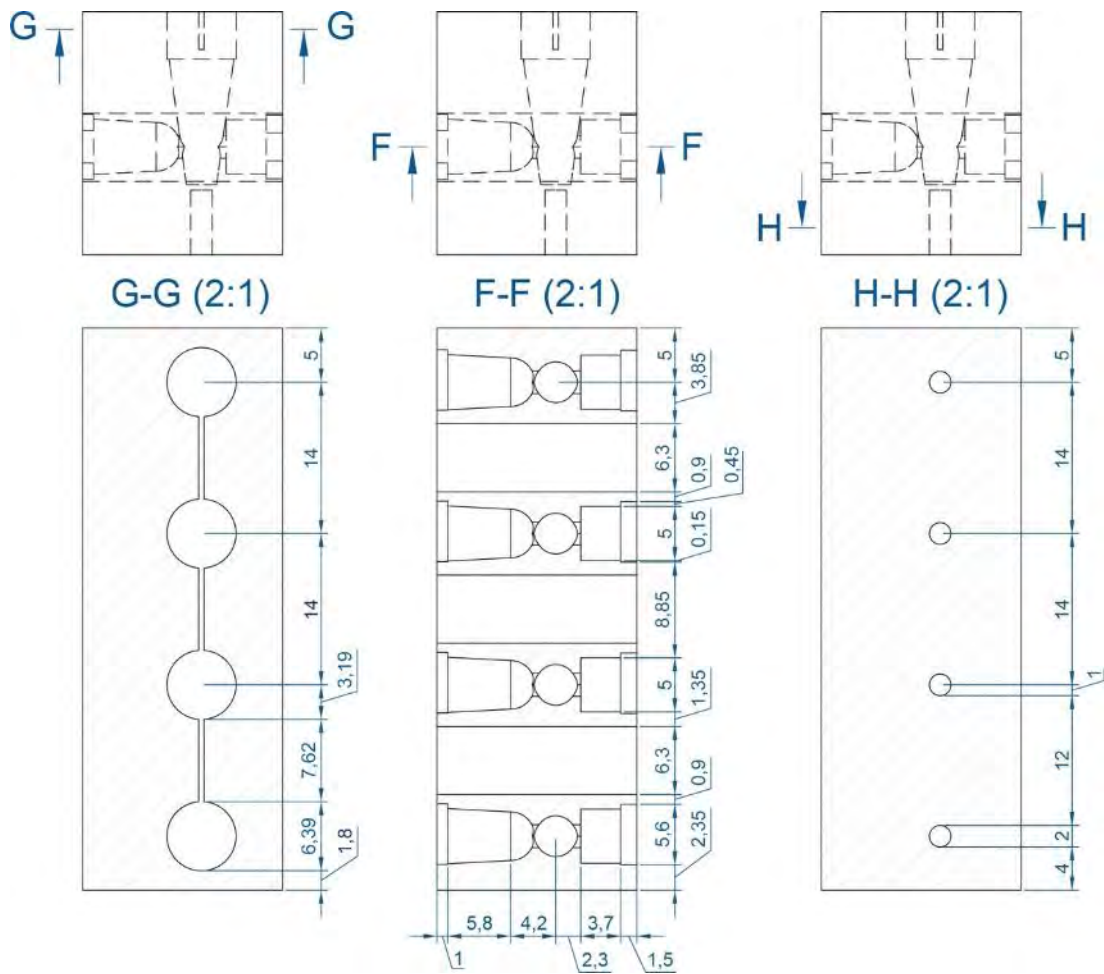
Figure S9 – 3D printed device model and a single slice of the device, slice F-H. The figure includes device dimensions and components that attach to the device. Millimetre measurements.
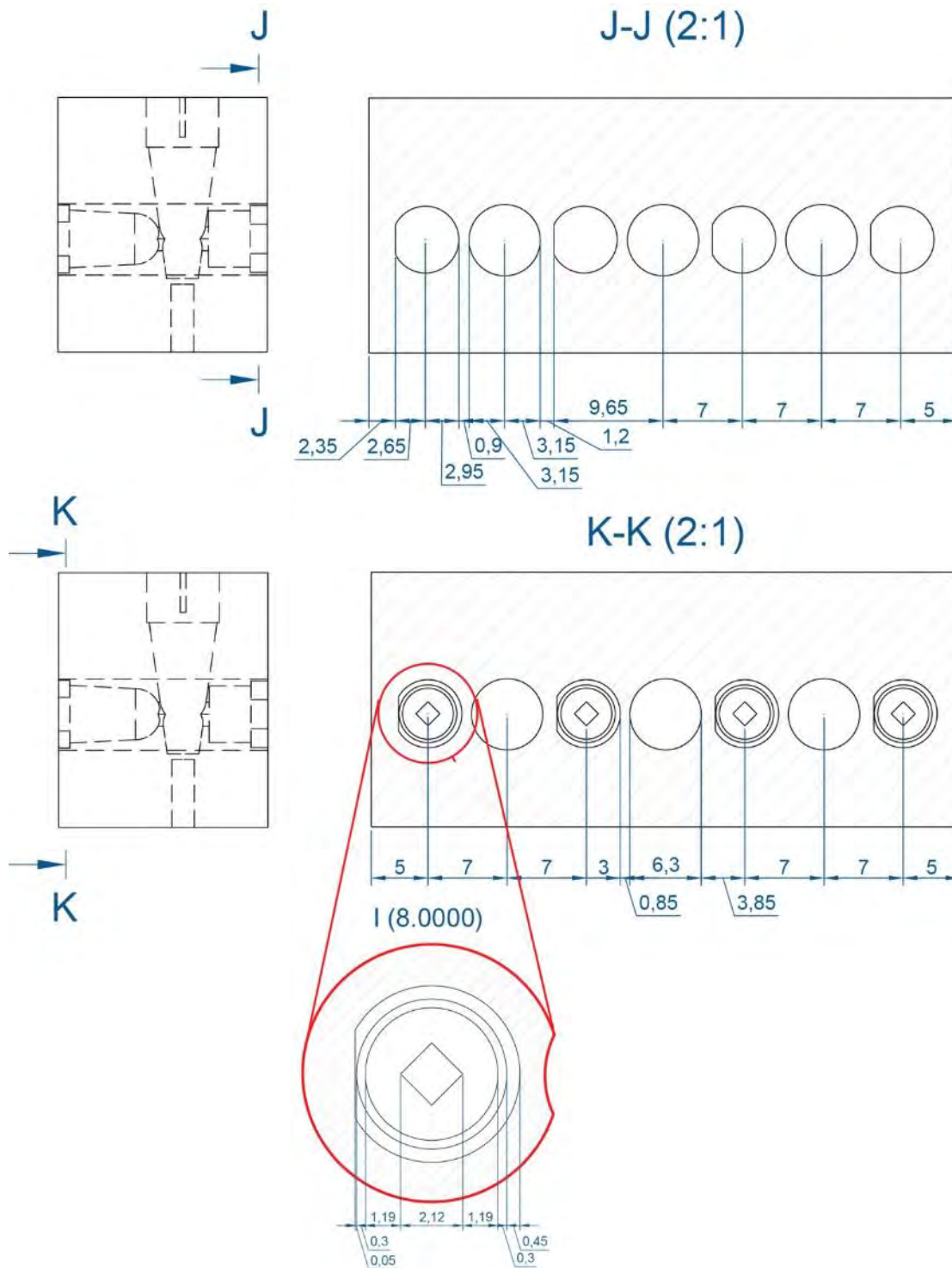
Figure S10 – 3D printed device model and a single slice of the device, slice J-K. The figure includes device dimensions and components that attach to the device. Millimetre measurements.
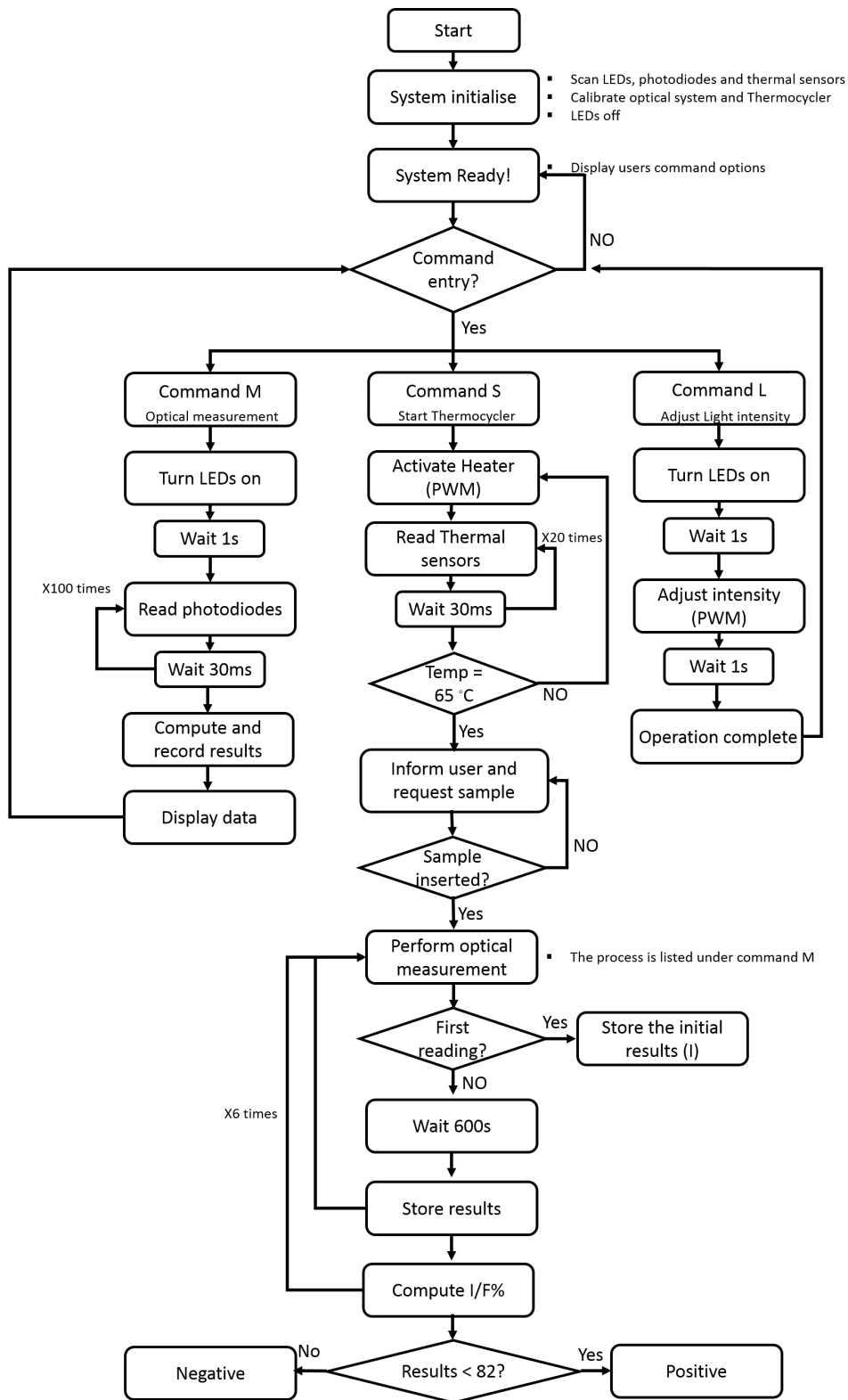
Figure S11. System firmware flow chart. This provides the complete unit operation for the portable diagnostic system to control the heating unit to amplify the patient samples and to analyze the turbidity indicting either positive or negative results ◦.
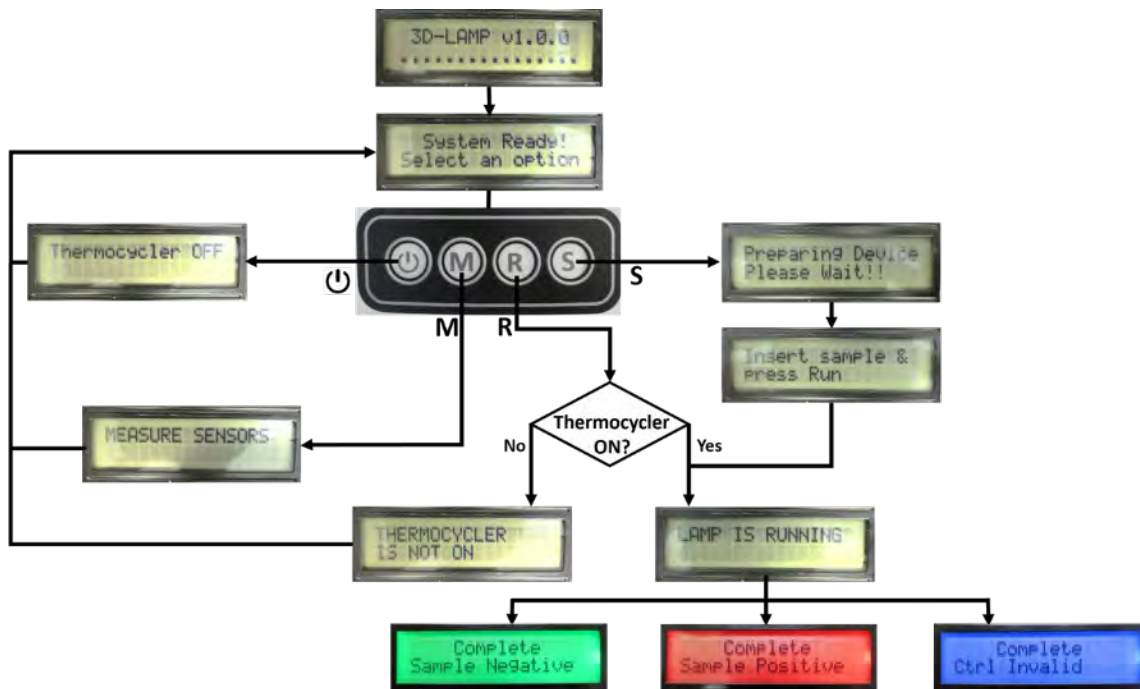
Figure S12 – **Display functionality flow chart**. This is a visual representation of the different stages of the LAMP reaction and the displayed feedback seen by the user.

```
....................... Initialising system ....................
Sensor 0 is: 260
Sensor 1 is: 278

Therm 0 is: 947
Therm 1 is: 952
 ....................          System Ready!       ...............
 ...........................................................................
Please choose one of the following commands:
-  Enter S to start the thermocycler
-  Enter M to Measure photosensors and thermistors
-  Enter L to Adjust the light intensity on LEDs (The format is L PWM_value e.g. L 125)
 ...........................................................................
Preparing Device. Please Wait!!

 .........................................
Current temperture is: 63
Insert Sample and enter R to continue

Measured at 600 Seconds

Sample1 is NEAGTIVE  96.47%
Sample2 is NEAGTIVE  102.04%

Measured at 1200 Seconds

Sample1 is NEAGTIVE  102.75%
Sample2 is NEAGTIVE  111.41%

Measured at 1800 Seconds

Sample1 is NEAGTIVE  106.67%
Sample2 is NEAGTIVE  93.06%

Measured at 2400 Seconds

Sample1 is NEAGTIVE  101.18%
Sample2 is POSITIVE  72.65%

Measured at 3000 Seconds

Sample1 is NEAGTIVE  101.57%
Sample2 is POSITIVE  70.20%

Measured at 3600 Seconds

Sample1 is NEAGTIVE  107.45%
Sample2 is POSITIVE  76.73%

Measurement Complete.
..... System Shutting Down!! ......|
```

Figure S13 – Representative read out of a sample that is negative.

```
...................... Initialising system ....................
Sensor 0 is: 322
Sensor 1 is: 369

Therm 0 is: 977
Therm 1 is: 977
 ...................            System Ready!        ...............
 ............................................................
Please choose one of the following commands:
-  Enter S to start the thermocycler
-  Enter M to Measure photosensors and thermistors
-  Enter L to Adjust the light intensity on LEDs (The format is L PWM_value e.g. L 125)
 ..............................................................
Preparing Device. Please Wait!!
.....................................

Current temperture is: 64.7
Insert Sample and enter R to continue

Measured at 600 Seconds

Sample1 is NEGATIVE  95.04%
Sample2 is NEGATIVE  91.77%

Measured at 1200 Seconds

Sample1 is NEGATIVE  91.84%
Sample2 is NEGATIVE  82.93%

Measured at 1800 Seconds

Sample1 is NEGATIVE  97.87%
Sample2 is NEGATIVE  93.90%

Measured at 2400 Seconds

Sample1 is NEGATIVE  98.58%
Sample2 is NEGATIVE  96.04%

Measured at 3000 Seconds

Sample1 is NEGATIVE  93.26%
Sample2 is NEGATIVE  86.59%

Measured at 3600 Seconds

Sample1 is NEGATIVE  95.74%
Sample2 is NEGATIVE  91.46%
Measurement Complete.
..... System Shutting Down!! ......
```

Figure S14 – **Representative read out of a sample that is invalid.** This is due to the internal positive control failing to amplify.

```
.......................... Initialising system .....................
Sensor 0 is: 206
Sensor 1 is: 177

Therm 0 is: 967
Therm 1 is: 965
 ......................           System Ready!        ................
 ..................................................................
Please choose one of the following commands:
-  Enter S to start the thermocycler
-  Enter M to Measure photosensors and thermistors
-  Enter L to Adjust the light intensity on LEDs (The format is L PWM_value e.g. L 125)
 .....................................................................
Preparing Device. Please Wait!!
 ........................................
Current temperture is: 869
Insert Sample and enter R to continue

Measured at 600 Seconds

Sample1 is NEAGTIVE  97.44%
Sample2 is NEAGTIVE  95.54%

Measured at 1200 Seconds

Sample1 is NEAGTIVE  95.24%
Sample2 is NEAGTIVE  93.31%


Measured at 1800 Seconds

Sample1 is POSITIVE  72.89%
Sample2 is POSITIVE  77.32%

Measured at 2400 Seconds

Sample1 is POSITIVE  68.50%
Sample2 is POSITIVE  66.17%

Measured at 3000 Seconds

Sample1 is POSITIVE  68.13%
Sample2 is POSITIVE  63.20%

Measured at 3600 Seconds

Sample1 is POSITIVE  69.60%
Sample2 is POSITIVE  64.31%

Measurement Complete.
..... System Shutting Down!! ......
```

Figure S15 - Representative read out of a sample that is positive. This is due to the internal positive control failing to amplify

14
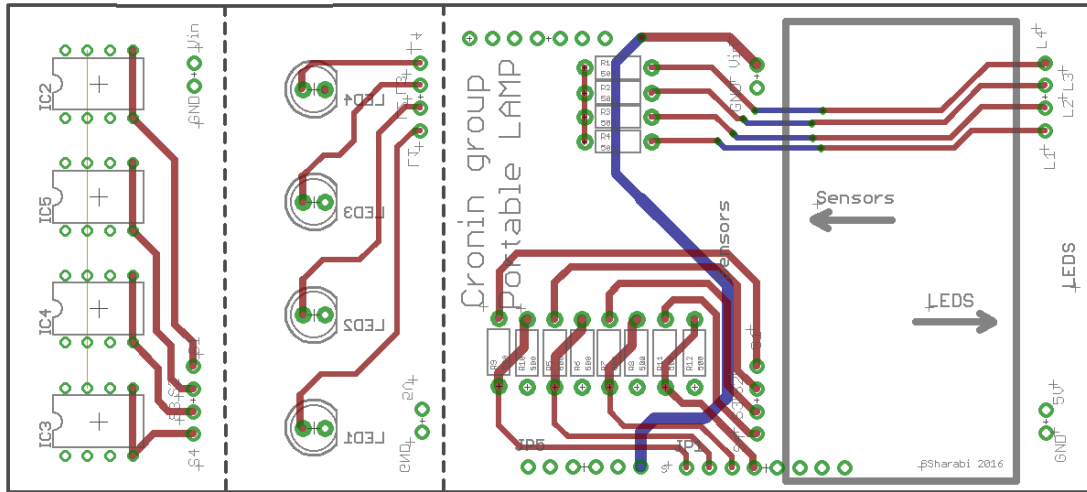
Figure S16 – **Complete layout for APTEC assay detection system for malaria samples.**
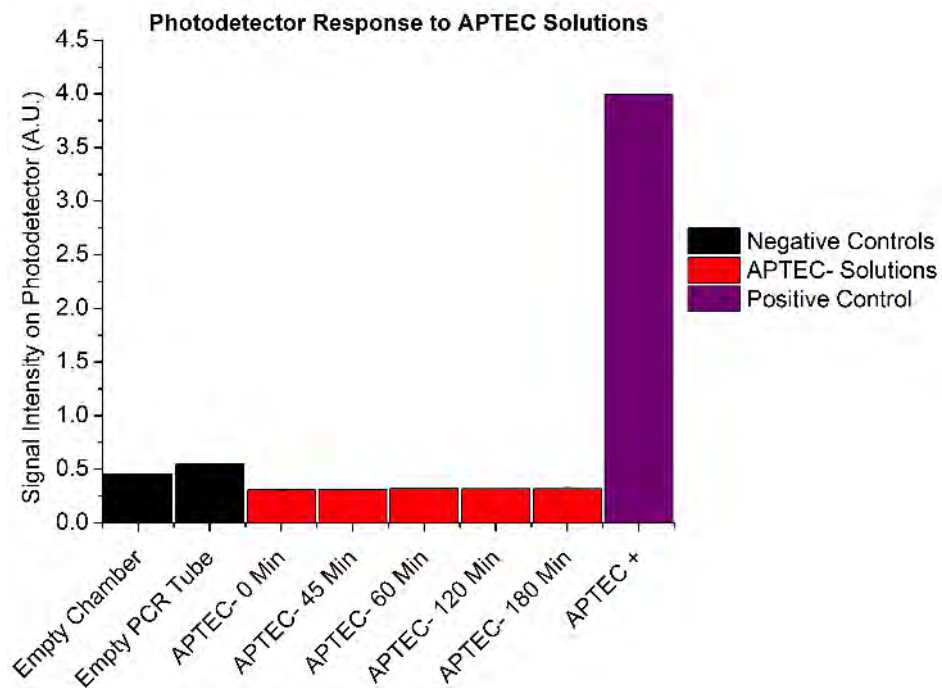
Figure S17 - Device-mediated detection of positive and sample assays in malaria APTEC assay. The portable device was assessed in its ability to distinguish a standard positive APTEC signal from its background and negative samples. Triplicate negative samples (APTEC-) were incubated for up to 180mins.
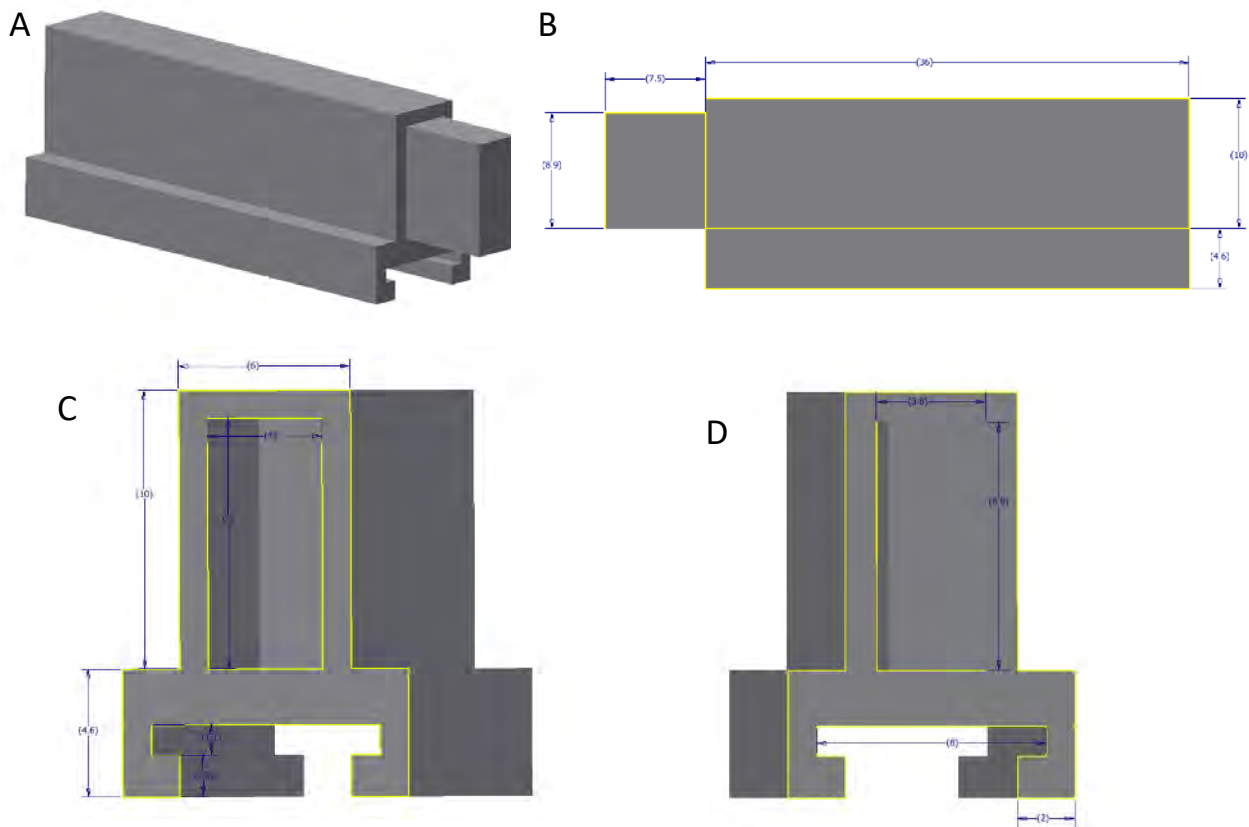
Figure S18. **3D Schematic of handle of magnetic transfer device.** A- image of handle. B – Side view of handle. C- Handle to Handle mortise. D – Handle tenon. All measurements are in mm.
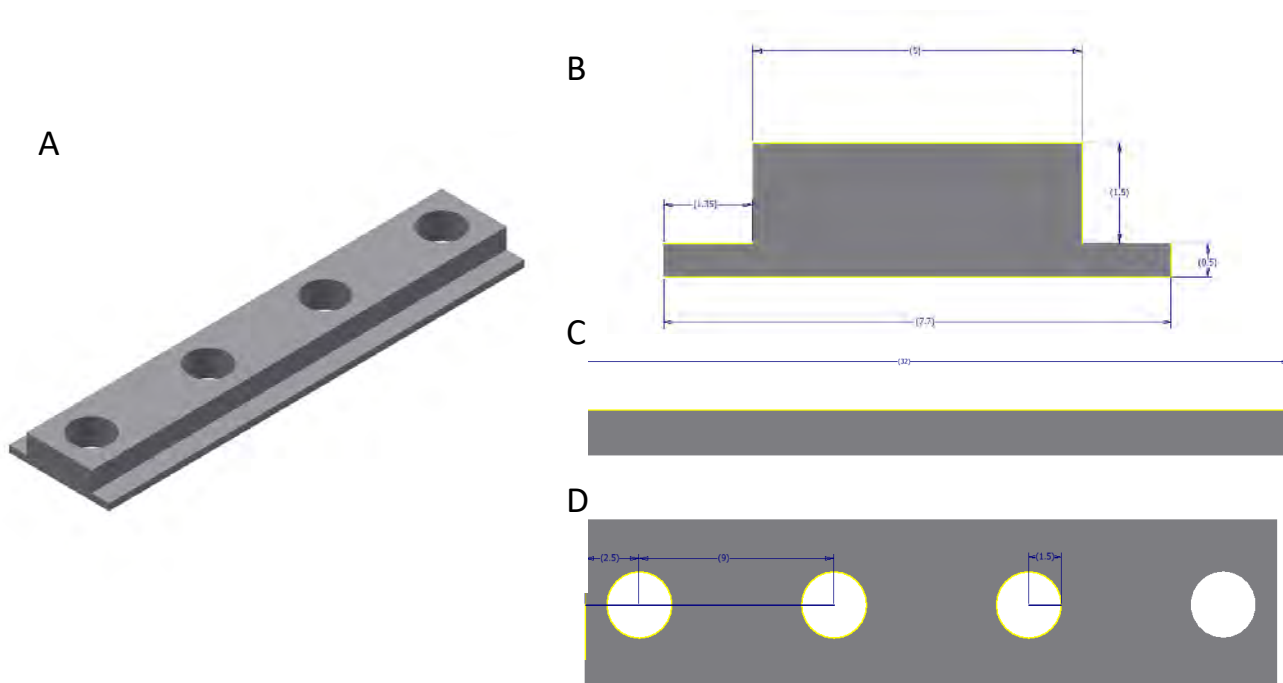
Figure S19. **3D schematic of APTEC assay magnet holder insert for handle.** A - top view of magnet insert. Rounded magnets are placed into the evenly spaced holes. B – end view of of magnet holder. C – Side view of magnet holder. D – Bottom view of magnet holder.
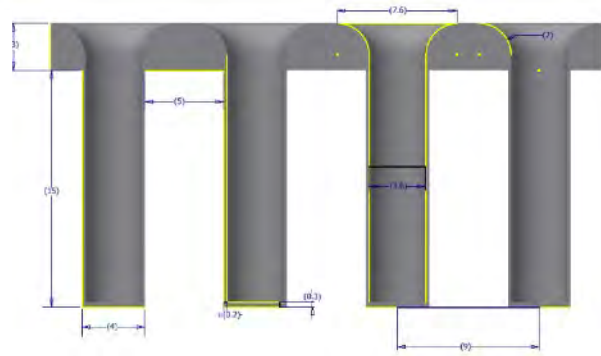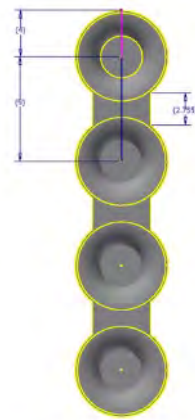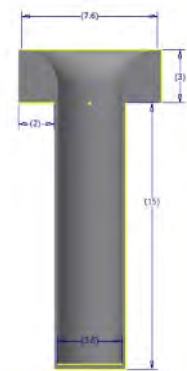
Figure S20. **3D schematic of APTEC assay magnet Sheath.** A – 3D image of magnet sheath. B- Side view schematic of magnetic sheath. C – Top view schematic of magnetic sheath. D – Side end-on view of magnetic sheath.
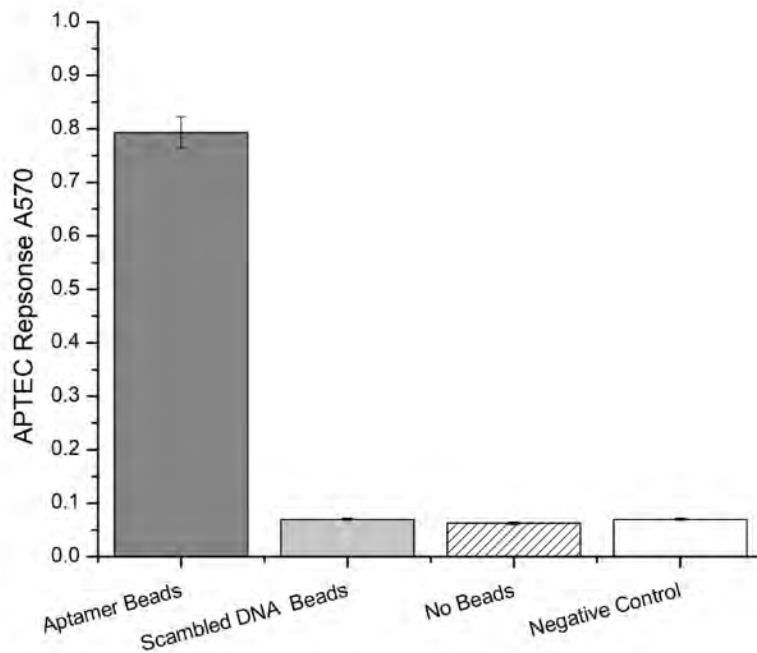
Figure S21: Confirming specificity of APTEC assay magnetic transfer tool. To confirm that the tool did not non-specifically capture the target enzyme we ran the APTEC assay with a positive blood sample using beads with scrambled aptamers and without beads. The positive control used aptamer beads and the negative control was performed on blood that did not contain the malaria enzyme.
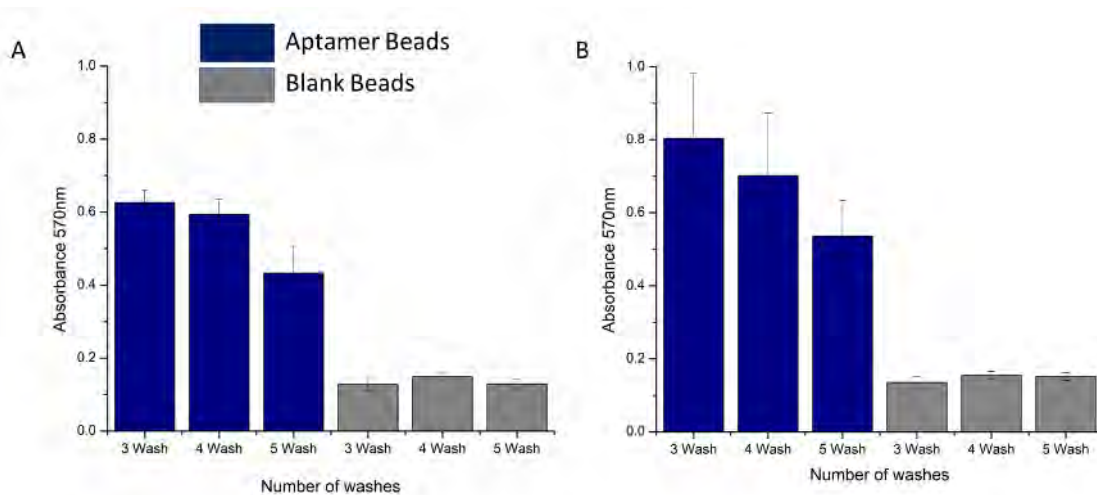
Figure S22: Optimisation of APTEC assay wash steps and development protocol. Full APTEC assays in positive samples were performed in triplicate. 3 washes were the number required to remove all visible blood debris from magnetic beads. A – Represents a comparison of aptamer coated magnetic beads with blank beads magnetic beads in which the magnetic transfer tool is not removed from the development solution. B – Represents a comparison of aptamer coated magnetic beads with blank beads magnetic beads in which the magnetic transfer tool is removed from the development solution.

Cost Comparison		
Portable LAMP Device		
	3D printed component	$4.70
	Custom PCB	$19.44
	Arduino board	$43
	Total	$67.14
Portable Malaria Device		
	3D printed component	$4.56
	Custom PCB	$13.31
	Arduino board	$43
	Total	$60.87
Laboratory RT-PCR		
	Commercial RT PCR Instrument	>$20,000
	Total	
Laboratory Plate Reader		
	Commercial Benchtop plate reader	>$4,000
	Total	

Table S1: Cost breakdown of portable devices versus laboratory devices.

Source Code

```
/* ==================================================================



//                      Time-stamp: <Wed Jun 05 11:38:03 GMT Standard Time 2015>
//  3DLAMP.ino
//
//  by Salah Sharabi, Cronin Group, University of Glasgow
//
//  Description:
//  Firmware for 3D-LAMP board
//----------------------------------------
//  The resistive heating Proportional circuit:
   * Heater 1 switch enable to digital pin 7
   * Heater 2 switch enable to digital pin 8
   * Heater 3 switch enable to digital pin 9
//----------------------------------------
//  The LCD circuit:
   * LCD RS pin to digital pin 47
   * LCD Enable pin to digital pin 45
   * LCD D4 pin to digital pin 43
   * LCD D5 pin to digital pin 41
   * LCD D6 pin to digital pin 39
   * LCD D7 pin to digital pin 37
   * LCD R/W pin to ground
   * 10K variable resistor
   * ends to +5V and ground
//----------------------------------------
//  The Optical sensing circuit:
   * LED1 pin to digital pin 10
   * LED2 pin to digital pin 11
   * LED3 pin to digital pin 12
   * LED4 pin to digital pin 13
   * Photosensor1 pin to analog pin A0 through an amplifier with a gain of 2 (Av= -20K/10K)
   * Photosensor2 pin to analog pin A1 through an amplifier with a gain of 2 (Av= -20K/10K)
   * Photosensor3 pin to analog pin A2 through an amplifier with a gain of 2 (Av= -20K/10K)
   * Photosensor4 pin to analog pin A3 through an amplifier with a gain of 2 (Av= -20K/10K)
//----------------------------------------
//  The thermal sensing circuit:
   * Thermestor1 pin to analog pin A4 through an RC filter with a cutoff frequency of 5Hz
   * Thermestor2 pin to analog pin A5 through an RC filter with a cutoff frequency of 5Hz
   * Thermestor3 pin to analog pin A6 through an RC filter with a cutoff frequency of 5Hz
   * Thermestor4 pin to analog pin A7 through an RC filter with a cutoff frequency of 5Hz
//----------------------------------------
//  The one wire Keypad circuit:
   * Keypad readout pin is connected to analog pin A8
    The Keypad consists of 4 Keys
              VDD
               |
              R=2K
```

```
                |
        -----Key1 -------- to analog pin
        |          |
        |       R=330
        |          |
        |-----Key2 ----
        |          |
        |       R=660
        |          |
        ------Key3 ----
        |          |
        |       R=1K
        |          |
        ------Key4 ----
        |
      GND
//**********************************************************************
*/
// include the library code:
#include <LiquidCrystal.h>
#include <SerialCommand.h>

// define the number of iteration for sensor measurement
int numIteration=100;        // Number of measurement iteration

// define the required variable for LCD dispaly
// initialize the library with the numbers of the interface pins
LiquidCrystal lcd(47, 45, 43, 41, 39, 37);
// LCD readout pin
int OneWireKeypadPin = A8;   // select the input pin for the potentiometer
//LCD readout variable
int KeypadValue = 0;         // variable to store the value coming from the keypad
// LCD backlight pins
int RedEnPin = 35;           // select the pin for the RED LCD background
int GrnEnPin = 33;           // select the pin for the GREEN LCD background
int BluEnPin = 31;           // select the pin for the BLUE LCD background

double InitialVal[4];        // array to store the photodiode nitial results (During sample mesaurmeents)
double FinalVal[4];          // array to store the photodiode Final results (During sample mesaurmeents)
double MeasVal[4];           // array to store the photodiode results (this array is used for diagnostics only)
double Result[4];            // array to store computed results = Final/Initial (During sample mesaurmeents)

int SysReady = 0;
int SecondsTimer, NestedTimer =0;
int LED;
int InitialMeas = 0;
int RunSample = 0;
int lightLevel= 0;
int Startup = 0;

bool InvalidTest = 0;
bool PositiveTest = 0;
bool NegativeTest = 0;
```

```
int PWMtemp = 125;          // 50% duty cycle
int Heater1 = 7;         // Heater 1 pin 7
int Heater2 = 8;         // Heater 2 pin 8
int Heater3 = 9;         // Heater 3 pin 9


int T1 = 4;
int T2 = 5;
int T3 = 6;
int T4 = 7;

int tempVal1;
int tempVal2;

// definite loop variables
int c, i, j, n, m, s ;

const int MaxThreshold = 870;    // a threshold level that's represents 63 degree C this is obtained from the
thermistors calibration table
//const int MinThreshold = 900;  // an arbitrary threshold level that's in the range of the analog input
// SerialCommand object
SerialCommand sCmd;

void SystemInit(void){
/*
   This is the system init routine
   This is run exactly once, when the Arduino reset (reset button, power on, new firmware).
*/
//**************************************************
// For Photo-sensor readings. All pins to input
//**************************************************
   pinMode(A0, INPUT);
   pinMode(A1, INPUT);
   pinMode(A2, INPUT);
   pinMode(A3, INPUT);
//***************************************************
// Thermal sensors
//**************************************************
   pinMode(A4, INPUT);
   pinMode(A5, INPUT);
   pinMode(A6, INPUT);
   pinMode(A7, INPUT);
//**************************************************
// For one wire Keypad
//**************************************************
   pinMode(A8, INPUT);
//**************************************************
// For Heat control (resistve heaters switchs)
//**************************************************
   pinMode(7, OUTPUT);
   pinMode(8, OUTPUT);
   pinMode(9, OUTPUT);
//**************************************************
```

```
// For LED control
//*****************************************************
   pinMode(10, OUTPUT);
   pinMode(11, OUTPUT);
   pinMode(12, OUTPUT);
   pinMode(13, OUTPUT);
//*****************************************************
// For LCD Display control (for RGB display only)
//*****************************************************
   pinMode(31, OUTPUT);   // Enable BLUE background
   pinMode(33, OUTPUT);   // Enable GREEN background
   pinMode(35, OUTPUT);   // Enable RED background
//*****************************************************
// Setup serial Communication speed. Diagnostics only
//*****************************************************
  Serial.begin(9600);
//*****************************************************
// Setup LCD's number of columns and rows: (16 columns and 2 rows)
//*****************************************************
  lcd.begin(16, 2);
//*****************************************************
   sCmd.setDefaultHandler(unrecognized); // Handler for command that isn't matched (says "What?")
   // abbreviated commands
   sCmd.addCommand("M", ReadSensorAll);
   sCmd.addCommand("R", MeasureSample);
   sCmd.addCommand("L", LightCtrl);
   sCmd.addCommand("S", StartThermocycler);
//*****************************************************
// TIMER SETUP
// the timer interrupt allows precise control switch
// for more info about configuration of Arduino timers see http://arduino.cc/playground/Code/Timer1
//*****************************************************
   cli();//Disable interrupts

   //set timer1 interrupt at 1Hz
   TCCR1A = 0;        // set entire TCCR1A register to 0
   TCCR1B = 0;        // same for TCCR1B
   TCNT1  = 0;        //initialize counter value to 0;
   // set compare match register for 1Hz increments
   OCR1A = 15624;       // = (16*10^6) / (1*1024) - 1 (must be <65536)
   // turn on CTC mode
   TCCR1B |= (1 << WGM12);
   // Set CS12 and CS10 bits for 1024 prescaler
   TCCR1B |= (1 << CS12) | (1 << CS10);
//  Do not enable timer compare interrupt yet.
//  It should be enabled when you want the timer to start ticking
//  TIMSK1 |= (1 << OCIE1A);

   sei();        //allow interrupts
}

// This gets set as the default handler, and gets called when no other command matches.
void unrecognized(const char *command) {
```

```arduino
    Serial.println("Invalid Command! Please try again");
  }

void LightCtrl() {
  int pwmNum;
  char *arg;
   // read light level
  arg = sCmd.next();
  if (arg != NULL) {
   for (j = 0; j <=3; j++) {
     pwmNum=j+10;
     lightLevel = atoi(arg); // Converts a char string to an integer
     analogWrite(pwmNum, lightLevel);
   }
  }
  Serial.println("LED intensitiy changed!");
   Serial.println("------------------------");
}

void StartThermocycler(){
 // Print a message to the LCD.
 lcd.clear();          // Clear Display
 lcd.setCursor(0,0);      // Set curser to pixel (0,0) column 0 row 0
 lcd.print("Preparing Device.");
 lcd.setCursor(0,1);      // Set curser to pixel (0,1) column 0 row 1
 lcd.print("Please Wait!! ");
 Serial.println("Preparing Device. Please Wait!! ");
 // enable timer compare interrupt
   TIMSK1 |= (1 << OCIE1A);
 }

void DisableBackLight(){
 digitalWrite(RedEnPin, LOW);  // turn RED background off
 digitalWrite(GrnEnPin, LOW);  // turn Green background off
 digitalWrite(BluEnPin, LOW);  // turn Blue background off
 delay(50);
}

void SamplePositiveDisp(int s){
 DisableBackLight();
 delay(10);
 digitalWrite(RedEnPin, HIGH);  // set background light to RED
// Print a message to the LCD.
 lcd.clear();           // Clear Display
 lcd.setCursor(0,0);       // Set curser to pixel (0,0) column 0 row 0
 lcd.print("TEST POSITIVE");
 lcd.setCursor(0,1);       // Set curser to pixel (0,1) column 0 row 1
 lcd.print("Sample");
 lcd.setCursor(8,1);
 lcd.print (Result[s]);
 lcd.setCursor(12,1);
 lcd.print ("%");
}
```

```
void SampleNegativeDisp(int s){
 DisableBackLight();
 delay(10);
 digitalWrite(GrnEnPin, HIGH);  // set background light to GREEN
 lcd.clear();              // Clear Display
 lcd.setCursor(0,0);       // Set curser to pixel (0,1) column 0 row 0
 lcd.print("TEST NEGATIVE");
 lcd.setCursor(0,1);       // Set curser to pixel (0,1) column 0 row 1
 lcd.print("Sample");
 lcd.setCursor(8,1);
 lcd.print (Result[s]);
 lcd.setCursor(12,1);
 lcd.print ("%");
}

void SampleInvalidDisp(int s){
 DisableBackLight();
 delay(10);
 digitalWrite(BluEnPin, HIGH);  // set background light to BLUE
 lcd.clear();              // Clear Display
 lcd.setCursor(0,0);       // Set curser to pixel (0,1) column 0 row 0
 lcd.print("INVALID TEST");
 lcd.setCursor(0,1);       // Set curser to pixel (0,1) column 0 row 1
 lcd.print("Control");
 lcd.setCursor(8,1);
 lcd.print (">82");
}

void SamplePositivFinal(){
 DisableBackLight();
 delay(10);
 digitalWrite(RedEnPin, HIGH);  // set background light to RED
// Print a message to the LCD.
 lcd.clear();              // Clear Display
 lcd.setCursor(0,0);       // Set curser to pixel (0,0) column 0 row 0
 lcd.print("  Complete   ");
 lcd.setCursor(0,1);       // Set curser to pixel (0,1) column 0 row 1
 lcd.print("Sample Positive");
}

void SampleNegativeFinal(){
 DisableBackLight();
 delay(10);
 digitalWrite(GrnEnPin, HIGH);  // set background light to GREEN
 lcd.clear();              // Clear Display
 lcd.setCursor(0,0);       // Set curser to pixel (0,1) column 0 row 0
 lcd.print("  Complete   ");
 lcd.setCursor(0,1);       // Set curser to pixel (0,1) column 0 row 1
 lcd.print("Sample Negaitive");
}

void SampleInvalidFinal(){
```

```
   DisableBackLight();
   delay(10);
   digitalWrite(BluEnPin, HIGH);  // set background light to BLUE
   lcd.clear();              // Clear Display
   lcd.setCursor(0,0);          // Set curser to pixel (0,1) column 0 row 0
   lcd.print("  Complete  ");
   lcd.setCursor(0,1);          // Set curser to pixel (0,1) column 0 row 1
   lcd.print("Ctrl Invalid");
}

int GetKey(void){
  int KeyValue;
  int x = analogRead (8);      // Read analog input A8 ands store the value in x
  delay(100);            // switch debounce
  x = analogRead (8);      // update x
  if (x < 60) {
    KeyValue = 1;            // if x < 60 set Key value to 1 (Start thermocycler)
  }
  else if (x < 200) {
    KeyValue = 2;            // if x < 200 set Key value to 2 (Run LAMP)
  }
  else if (x < 400){
    KeyValue = 3;            // if x < 400 set Key value to 3 (Measure sensors)
  }
  else if (x < 600){
    KeyValue = 4;            // if x < 600 set Key value to 4 (Stop)
  }
  return KeyValue;
}

void LEDoff(){
 // Loop to turn all 4 LEDs off
 for (j = 0; j <= 3; j++) {
   // LED variable is used as a variable to address the LED pins
   LED = j + 10;          // LED is offset by 10
   digitalWrite(LED,HIGH);   // Set LED (10,11,12,13) High (off)
   delay (50);           // the usual wait to stabilise
  }
}

void LEDon(){
 // Loop to turn all 4 LEDs on
 for (j = 0; j <= 3; j++) {
   // LED variable is used as a variable to address the LED pins
   LED = j + 10;            // LED is offset by 10
   digitalWrite(LED,lightLevel); // Set LED (10,11,12,13) LOW (On)
   delay (50);              // the usual wait to stabilise
  }
}

void HeatersOff(){
 digitalWrite(Heater1,LOW);  // Enable resistive heater1 ON. Switch transistor on with 5V on the gate terminal
 digitalWrite(Heater2,LOW);  // Enable resistive heater2 ON. Switch transistor on with 5V on the gate terminal
```

```arduino
    digitalWrite(Heater3,LOW);  // Enable resistive heater3 ON. Switch transistor on with 5V on the gate terminal
}

void SensorsTest(){
  int out;
  // Diagnostic: check if all LED are on before you run any measurements
  LEDon();          // Turn LEDs ON
  delay(100);       // Wait
  MeasPins();       // Measure to make sure they are all on
//---------------------------------------------
// Diagnostic only (Serial print on a computer screen)
  for (j = 0; j <=3; j++) {
    // Store the measured results in a temporary array
    out = MeasVal[j];
    Serial.print("Sensor A"); Serial.print(j); Serial.print(" is: ");
    Serial.println(out, DEC);
  }
//---------------------------------------------
  LEDoff();         // Turn LEDs OFF
}

void ThermTest(){
  int out;
  // Diagnostic: check if all thermistors are on before you run any measurements
  MeasPins();       // Measure to make sure they are all on
//---------------------------------------------
// Diagnostic only (Serial print on a computer screen)
  for (j = 4; j <=7; j++) {
    // Store the measured results in a temporary array
    out=MeasVal[j];
    Serial.print("Therm A"); Serial.print(j); Serial.print(" is: ");
    Serial.println(out, DEC);
  }
//---------------------------------------------
}

void LEDTest(){
// Another Diagnostic: startup sequense; LEDs OFF then ON then OFF again
// just to make sure that all LEDs are funcrional
  for (j = 0; j <= 3; j++) {
    LED = j + 10;
    digitalWrite(LED,HIGH);       // LEDs OFF
    delay (100);
    digitalWrite(LED,LOW);        // LEDs ON
    delay (100);
    digitalWrite(LED,HIGH);       // LEDs OFF
    delay (100);
  }
}

void ReadSensorAll(){
  LEDon();                  // Turn ON all LEDs
  delay(100);               // wait till stable to avoid flickering
```

```arduino
    MeasPins();              // measure all sensors, thermal and optical.
//-------------------------------------------
// Diagnostic only
  for (i = 0; i < 8; i++) {
    Serial.println( MeasVal[i], DEC);
  }
  Serial.println("---------------------");
//-------------------------------------------
  LEDoff();                // turn LEDs off
  lcd.clear();             // Clear Display
  lcd.setCursor(4,0);
  lcd.print("Complete");        // Display Complete
  delay(5000);             // keep for 5 seconds
  LoopMessage();              // Display the "Ready" message
}

void MeasureSample(){
  LEDon();                 // Turn ON all LEDs
  delay(100);
  MeasPins();                // measure all optical sensors
  for (i = 0; i <=3; i++) {
   if (InitialMeas==0){
    // return averaged value
    InitialVal[i] = MeasVal[i];  // Store the first measured value for all the samples
    Serial.print("I");Serial.print(i+1);Serial.print(" is:"); Serial.println(InitialVal[i]);
   }else{
    FinalVal[i] = MeasVal[i];    // if this is the first measurement store the results in a final value array
                      //that keeps updating till LAMP is complete
    Serial.print("F");Serial.print(i+1);Serial.print(" is:"); Serial.println(FinalVal[i]);
   }
  }
  Serial.println("");
  InitialMeas = 1;            // update variable initial (this variable is used to diffrentiate between the first ever
reading and any other reading that follows.
  LEDoff();                // turn LEDs off
}

void MeasPins(){
 for (j = 0; j <=7; j++) {
  // initialise buffer variable
  unsigned long voltagebuff = 0;
  analogRead(54+j);         // read offset pins // pin assignments are A0=54, A1=55, A2=56 and so on.
  delay(5);
  // read sensor <numIteration> times
  for (n = 0; n <= numIteration; n++) {
   // Read from A0-A7.
   // pin assignments are A0=54, A1=55, and so on.
   //See c:/Program Files (x86)/Arduino/hardware/arduino/variants/mega/pins_arduino.h for details.
   voltagebuff += analogRead(54+j);
   delay(1);
  }
  delay(5);
  // return averaged value
```

```cpp
      MeasVal[j] = voltagebuff/numIteration ;  // average measured values
  }
}


void SysCompute(){
  for (s=0; s<4; s++){
    Result[s]=(FinalVal[s]/InitialVal[s])*100;   // Compute the final result for all the samples  R=F/I*100
    // check control samples for invalid test
    if (NestedTimer >= 3){                 // only perform this step after 1800 seconds = 30min
      if (s==1 || s==3){                   // if we are reading the ctrl samples
        if (Result[s] >= 82){              // check for validity
          InvalidTest = 1;
          SampleInvalidDisp(s);            // Display invlaid ctrl message
          Serial.print("Control");Serial.print(s+1);Serial.print(" is ");Serial.print("INVALID
");Serial.print(Result[s]);Serial.println("%");
        }else{
          InvalidTest = 0;                 // Otherwise carry on
          Serial.print("Control");Serial.print(s+1);Serial.print(" is ");Serial.print("OK
");Serial.print(Result[s]);Serial.println("%");
        }
      }else{
        if (Result[s] <= 82){              // scan the sample for positive results
          PositiveTest = 1;
          NegativeTest = 0;
          SamplePositiveDisp(s);           // if so display positive
          Serial.print("Sample");Serial.print(s+1);Serial.print(" is ");Serial.print("POSITIVE
");Serial.print(Result[s]);Serial.println("%");
        }else{
          PositiveTest = 0;
          NegativeTest = 1;
          SampleNegativeDisp(s);           // otherwise display negative
          Serial.print("Sample");Serial.print(s+1);Serial.print(" is ");Serial.print("NEGATIVE
");Serial.print(Result[s]);Serial.println("%");
        }
      }
    }
  }
}


void DisplayWelcomeMessage(){
  lcd.setCursor(1,0);
  lcd.print("3D-LAMP v1.0.0");
  lcd.setCursor(0,1);
  lcd.print("................");
  if (Startup==0){
    Serial.println("                    3DPCR V1.0.0                    ");
    Serial.println("......................... Initialising system .....................   ");
    Serial.println(" .....................          System Ready!        ................    ");
    Serial.println(" .................................................................   ");
    Serial.println("Please choose one of the following commands:");
    Serial.println("- Enter S to start the thermocycler");
    Serial.println("- Enter M to Measure photosensors and thermistors");
    Serial.println("- Enter L to Adjust the light intensity on LEDs (The format is L PWM_value e.g. L 125)");
```

```cpp
    Serial.println(" ............................................................................");
    Startup++;
  }
 LEDTest();        // Run LEDs test
 HeatersOff();      // Turn heaters off
 SensorsTest();      // Test If all sensors are present and functional
 ThermTest();       //
}

void LoopMessage(){
  lcd.clear();
  lcd.setCursor(2,0);
  lcd.print("System Ready!");
  lcd.setCursor(0,1);
  lcd.print("Select an option");
}

void setup() {
  SystemInit();            // Ssytem startup
  DisplayWelcomeMessage();       // initilise and display welcome message
  delay(3000);
  LoopMessage();
}

void loop() {
  sCmd.readSerial();         // We don't do much, just process serial commands
  KeypadValue = GetKey();      // Read Keypad
  delay(50);
  switch (KeypadValue) {
    case 1:          // Start Thermocycler
      DisableBackLight();
      lcd.clear();
      lcd.setCursor(0,0);
      lcd.print("Thermocycler ON");
      StartThermocycler();
      break;
    case 2:
      DisableBackLight();    // Run LAMP under the following conditions:
      // only when thermocycler is already on and the temperature is reached
      if (RunSample != 0){
       lcd.clear();
       lcd.setCursor(0,0);
       lcd.print("LAMP Running ...");
       MeasureSample();
      }else{          // If the thermcycler has not been activitated yet
       lcd.clear();
       lcd.setCursor(0,0);
       lcd.print("THERMOCYCLER");
       lcd.setCursor(0,1);
       lcd.print("IS NOT ON!");
       delay(10000);
       LoopMessage();
      }
```

```
      break;
    case 3:               // Measure all sensors (optical and thermal)
      DisableBackLight();
  // Measure all sensors
    lcd.clear();
    lcd.setCursor(0,0);
    lcd.print("MEASURE SENSORS");
    ReadSensorAll();
    LoopMessage();
      break;
    case 4:               // System shut down
      DisableBackLight();
    lcd.clear();
    lcd.setCursor(0,0);
    lcd.print("Thermocycler OFF");
    cli();
    HeatersOff();
    delay(60000);
    LoopMessage();
      break;
  }
  if (NestedTimer == 6){    // safty shut down after 1 hour

   cli();                // disable interrupt and therfore heaters are off

  }
}

ISR(TIMER1_COMPA_vect) {          // Proportional control driven by system Interrupt at freq of 1Hz
                     // This is also used to measure control the heating elements
 //tempVal1 = analogRead(T1);       //get val of thermstr 1 (A4)
 tempVal2 = analogRead(T1);        //get val of thermstr 3 (A6)
 if (tempVal2 >= MaxThreshold){     //if tempreture is greater than the threshold value
  //analogWrite(Heater3,PWMtemp);   //Activate heater
  analogWrite(Heater1,PWMtemp);      //Activate heater
  delay (1);             //nop
  }else{
   //digitalWrite(Heater3,LOW);     //Turn heater off
   digitalWrite(Heater1,LOW);       //Turn heater off
   if (SysReady==0){            //If the system reached 63 degrees C then request sample insertion
     Serial.println("");Serial.print("Current temperture is: ");Serial.println(tempVal2); //print the tempreture
value
     Serial.println("Insert Sample and enter R to continue");
     lcd.clear();
     lcd.setCursor(0,0);
     lcd.print("Insert sample &");
     lcd.setCursor(0,1);
     lcd.print("press Run");
     InitialMeas = 0;          // set variable initialMeas to 0 so we can perform the initial sample measurement
     RunSample = 1;           // set variable  Runsample to 1 so we can perform the initial sample
measurement after insertion
     SysReady++;              // Increment the system ready variable so it doesnt request sample any more till
the current samples are measured.
```

```arduino
    }
  }
  if (InitialMeas != 0){          // once initial measurement is performed
    SecondsTimer++;              // Start a seconds timer.
    if (SecondsTimer == 600){    // perform a measurmeent every 10 minutes
      NestedTimer++;            // increment an internal counter to limit the count to an hour
      MeasureSample();
      SysCompute();             // Compute the results and check results validity
      SecondsTimer = 1;         // reset seconds counter for another 10 mins
      if (NestedTimer == 6){
        HeatersOff();           // System shut down sequence after 1 hour (turn off heaters and sisplay Complete
and Shutting down)
        DisableBackLight();
// When the system completes the process the device turns off but keeps the display of the results on the LCD
screen
        if(InvalidTest == 1){
          SampleInvalidFinal();   // This is for Invalid
        }else if(PositiveTest == 1){
          SamplePositivFinal();   // this is for Positive resutls
        }else if(NegativeTest == 1){
          SampleNegativeFinal();  // This is for Negative results
        }
        Serial.println("Measurement Complete.");
        Serial.println("..... System Shutting Down!! ......");
        cli();                  // Very very important to disable the interrupt
      }
    }
  }
}
```