Analytical Methods

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2018, **10**, 4671

Handheld battery-operated sample preparation device for qPCR nucleic acid detections using simple contactless pouring

Geoffrey Mulberry,[a] Alissa Vuillier,[a] Manjusha Vaidya,[b] Kiminobu Sugaya[b] and Brian N. Kim *[ab]

Received 3rd May 2018
Accepted 9th September 2018

DOI: 10.1039/c8ay00998h

rsc.li/methods

Sample preparation is an essential process that precedes nucleic acid detections which use quantitative polymerase chain reaction (qPCR). However, sample preparation is a labor-intensive process and requires skilled labor, thus limiting the public's access in low-resource settings to many high-quality nucleic acid-based detection mechanisms. In this paper, we present a simple, handheld, battery-operated sample preparation device to minimize user's involvement. The device uses a simple pouring method to process the DNA sample without pipetting or using disposable pipette tips. The developed device has a size of 12 × 8 × 8 cm^3 and mass of only 364 g. The device is compared to gold standard methods, including magnetic bead-based and silica filter-based DNA extractions. For a short segment DNA target of 68 bp, the presented device captured 8.67× more DNA compared to that of the manual magnetic bead-based method. Because of automation, the measured capture efficiency is more consistent and has a smaller deviation between multiple repetitions than the manual method. To present a comprehensive, portable, battery-operated diagnostic system, the sample preparation device is tested in conjunction with a 3D-manufactured qPCR device. The test using three diluted target DNA samples, each spiked in whole blood (1×, 0.1×, and 0.01×), revealed a quantitative detection with ideal cycle threshold separations between the measurements. The combination of two devices will aid in resource-limited settings to promptly and accurately diagnose infections of patients.

Introduction

Considering the emergence and persistence of infectious diseases such as malaria and Zika virus throughout the world, quantitative polymerase chain reaction (qPCR) processes have become increasingly useful to accurately test for these diseases and to also reveal the stage of infection by quantification.[1] However, alternative diagnostic methods are often used because qPCR requires a labor-intensive sample preparation process.[2] In a sample preparation process, nucleic acids (DNA and RNA) are extracted and purified from whole blood by removing other components of blood which can impede the nucleic acid detection mechanism. Sample preparation is involved in most diagnostic biochemical assays using whole blood.[3] When a person is speculated to be infected with pathogens by exhibiting common symptoms, their blood is drawn and transported to a centralized laboratory where the sample can be used for preparation and detection. Depending on the location, this process can take hours to days due to transportation and required labor. At the laboratory, the blood undergoes a sample preparation procedure, which involves many manual pipetting steps to perform blood lysis, DNA/RNA capture, washing, and elution (Fig. 1a) and thus can only be done by a skilled person at a specialized laboratory. Despite its limitations, this procedure has seen minimal changes in laboratory settings over the last two decades.[4] Therefore, simple, low-cost, and robust sample processing tools requiring no skilled labor are currently in demand to enable early diagnosis of infectious diseases.

Several approaches to simplifying PCR sample preparation have been proposed and tested in the past for rapid detection of diseases. A lab-in-a-drop system was developed to integrate point-of-care detection of nucleic acid biomarkers into a single fluid droplet.[5] A rotationally-driven microdevice integrated mixing capability with multiplexed PCR amplification to simply the design and increase efficiency, resulting in a novel and inexpensive PCR amplification system.[6] These approaches hold similarities in their attempts to lower costs and increase efficiency of sample preparation devices. Much recent research has moved more specifically in the direction of portable devices.[7] A portable room-temperature sample preparation system is pressure-driven for centrifuge-free nucleic acid extractions.[8] A chemical heater system was created to conduct rapid lysis to

[a]*Department of Electrical and Computer Engineering, College of Engineering and Computer Science, University of Central Florida, Orlando, USA. E-mail: brian.kim@ucf.edu*
[b]*Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, USA*

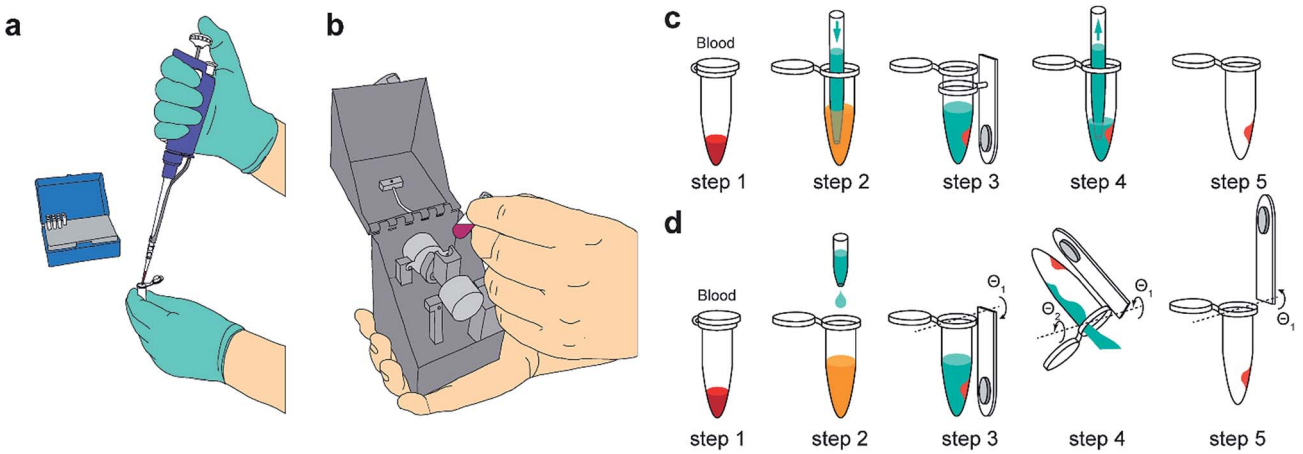

Fig. 1 Overview of the labor-intensive manual sample preparation and the automated sample preparation device. (a) Typical sample preparation requiring manual handling of the sample using pipettes and disposable pipette tips. (b) The handheld sample preparation device which autonomously processes whole blood. (c) The sample preparation using magnetic beads and manual pipettes: (step 1) adding whole blood sample, (step 2) adding washing buffer, (step 3) separating magnetic beads, (step 4) removal of supernatant liquid using pipette, and (step 5) resulting in isolated DNA. (d) Detailed operation of the automated method: (step 1) adding whole blood sample, (step 2) adding washing buffer using integrated contactless dispenser, (step 3) separating magnetic beads using a rotating magnet arm, (step 4) removal of supernatant liquid using contactless pouring, and (step 5) resulting in isolated DNA.

create a disposable system for increased convenience.[9] This approach to dry enzyme storage and sample heating was proven effective in amplifying both DNA and RNA, and was even capable of usage in low-resource laboratories. Additionally, electronic approaches have been studied in order to provide a hands-free sample preparation process. One system in particular integrates all the necessary agents for cell lysis, washing, and elution onto one chip to allow for total sample preparation to be handled at once.[10] Much research has strived for hands-free solutions, just as the device proposed in this paper is contact-free. Some research has utilized magnetic beads, particularly a magnetically actuated bead-beating system. One such system specifically performs sample lysis using the magnetic beads, before a centrifuging clarification step.[11] This enables the device to efficiently extract nucleic acid from biological samples. Similarly, a magnetic bead suspension can be used to trap magnets and release periodically. By rotating small permanent magnets, another device is able to conduct a washing cycle with high purification efficiency.[12] It is worth noting that irreversible bead aggregation can affect the DNA purification and the efficiency of diagnostic.[13] Often, the irreversible bead aggregation is caused by the overexposure to the magnetic force. It is important to precisely control the magnetic exposure to yield a reliable DNA extraction efficiency. Throughout all research in this area, integrating efficiency, portability, and effectiveness has been identified as a key priority for a novel PCR point-of-care sample preparation device.

In this paper, we present a simple, handheld, battery-operated sample preparation device designed to minimize a user's skilled labor (Fig. 1b). By simply inserting the sample, washing and DNA capture steps can be autonomously performed. To enable this, a simple pouring scheme is adapted. Manual sample preparation methods, as well as commercially-available automated sample preparation devices,[4] rely on pipetting to exchange and transfer liquids (Fig. 1c). The pipette uses disposable pipette tips which are designed for one-time use. Because pipette tips come in contact with the sample (Fig. 1c, step 2 and step 4), they need to be replaced after every usage to prevent contamination. In manual and commercial automated sample preparation methods which are based on magnetic bead-based separation, the blood lysis buffer and magnetic beads are added to whole blood (Fig. 1c, step 2), which promotes the binding of free DNA/RNA to magnetic beads. Beads with captured DNA molecules are accumulated by a magnet (Fig. 1c, step 3). The supernatant liquid is removed by pipetting (Fig. 1c, step 4), which results in a clump of magnetic beads. Step 2, 3, 4, and 5 are repeated to thoroughly wash the beads before using DNA for nucleic acid detection. Contactless pouring and dispensing to remove and add liquid can eliminate the need to replace disposable parts, like the pipette, and simplify the procedure. An overview of the pouring method is shown in Fig. 1d.

The process begins with raw sample from a patient (Fig. 1d, step 1), containing a large concentration of hemoglobin that is inhibitory to qPCR. This sample is placed into the automated sample preparation device (Fig. 1b). At the beginning of the process, a tube is loaded into the device containing the sample (Fig. 1f). This sample contains magnetic beads which bind to DNA in the sample. The tube motor then returns to the home position ($\theta_2 = 0°$) while the magnet motor simultaneously rotates to the downward position ($\theta_1 = 0°$). While in this state, the magnet is in a close proximity to the sample, attracting the magnetic beads to the tube wall. This step is ~30 seconds and results in a separation of magnetic beads by forming a bead pellet (separation step). To remove the supernatant before washing, both motors rotate simultaneously ($\theta_1 = \theta_2 > 100°$) so that the supernatant is poured into the waste reservoir, while

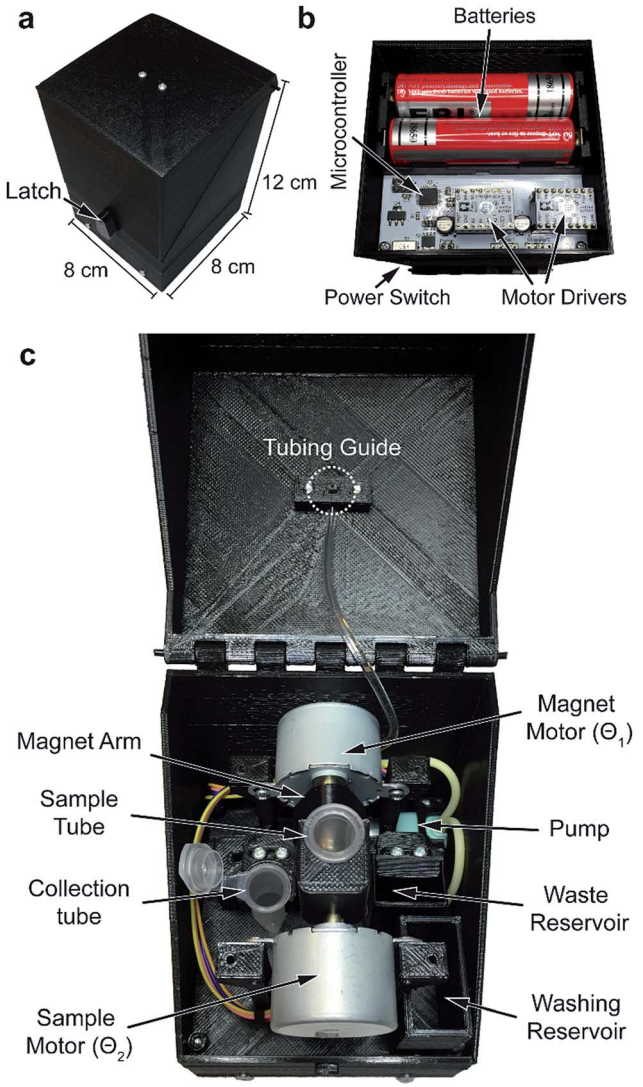

Fig. 2 Photographs of the completed sample preparation device. (a) The device with its lid closed, viewed from the front. (b) The bottom of the device with the lower cover removed, showing the circuit board and batteries for controlling and powering the device. (c) The device with the lid opened, viewed from the top. This shows the locations of integrated functional components, including the motors responsible for rotating the sample tube and magnet arms, as well as the pump and washing reservoir for delivery of washing buffer to the sample tube.

the magnetic beads are left attracted to the tube wall (pouring step). To add new washing buffer, the tube motor rotates the sample tube to the home position while the magnet moves away to the upward position ($\theta_1 = 180°$). Then, the peristaltic pump is enabled, dripping washing buffer from the washing reservoir to the sample tube (washing step). This step is followed by the resuspension of the magnetic beads by rotating the tube motor back and forth between $-90° < \theta_2 < 90°$ to gently remove the bead pellet off the tube wall (resuspension step). The process, consisting of separation, pouring, washing, and resuspension steps, repeats several times, repetitively purifying the DNA targets in every cycle. This sample can then be used in a real-time qPCR device to perform the PCR reaction and determine the level of infection in the patient's sample.

Experimental

The contactless sample preparation device is designed using the pouring scheme to enable the automation of the DNA/RNA extraction process without many disposable parts that need to be replaced often.

Device design and fabrication

The automated sample preparation device is designed to perform a magnetic bead-based DNA isolation from whole human blood. The mechanical design for the device was performed using Inventor 3D CAD software (Autodesk, San Rafael, CA). Parts for this device are fabricated using a 3D-printer for low-cost manufacturing at the prototyping stage. The 3D printed parts are printed with a 0.2 mm layer height at 200 °C at 60 mm s^{-1}. The device consists of nine 3D-printed parts, magnets, two stepper motors, a control circuit board, a pump, and two lithium-ion batteries. The design of the device is shown in Fig. 2.

The operation of the device involves the movement of the two stepper motors, one motor moving the magnet (θ_1) and the other moving the sample tube (θ_2). The position depicted in Fig. 1d, step 3 is when $\theta_1 = \theta_2 = 0°$. We used permanent magnets for the bead separation over an electromagnet to reduce power consumption. The small neodymium magnets produce a powerful magnetic field and have the added benefit of requiring no power to be consumed during their use, increasing battery life, an important consideration for a device designed for use at the point-of-care. The device is designed with integrated electronics to automate the sample preparation procedure by controlling the two stepper motors and peristaltic pump. An on-board microcontroller (Atmel ATmega328P) uses embedded programming to automate and time each step of the sample preparation. Two 3000 mA h lithium-ion 18 650 batteries are used for the power supply. The stepper motors are controlled through stepper motor drivers which take control signals from the microcontroller and supply power from the batteries to the motor coils. The peristaltic pump is controlled by the microcontroller using a MOSFET to turn on or off the DC motor on the pump.

Electronic and mechanical materials

The device is designed using filament-based 3D printing with polylactic acid (PLA) plastic as the filament. PLA was sourced from Hatchbox (Los Angeles, CA). Stepper motors (SKU 918) were sourced from Adafruit (New York, NY). The peristaltic pump (SKU RP-Q1.2N-P20A-DC3V) is from Aquatech (Osaka, Japan). All electronics were ordered from DigiKey (Thief River Falls, MN) except for the stepper motor controllers (item no. 2134) which were sourced from Pololu (Las Vegas, NV). The printed circuit board (PCB) was fabricated by PCBway (Hangzhou, China). The 18 650 batteries were from EBL (China).

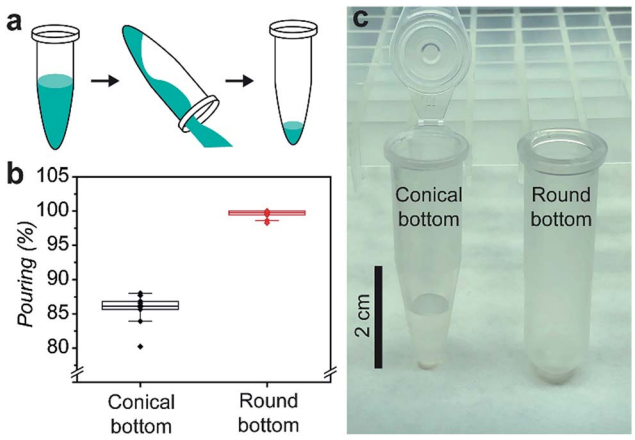

Fig. 3 The effective removal of liquid using conical-bottomed tube and round-bottomed tube. (a) A tube is used to pour out the water and remaining liquid due to surface tension is measured. (b) The pouring efficiency comparison between the conical-bottomed tube, 85.7 ± 0.67% (SEM), and round-bottomed tube, 99.52 ± 0.19% (SEM). (c) The photograph of two tubes after pouring out water showing retained water in the conical-bottomed tube.

Tubing (part no. ACF00001) was from Saint-Gobain (Paris, France).

Experimental setup

Because this project involves a new type of device, two different phases of experimentation are performed. The first phase involves qualification and optimization of the design itself, and the second phase involves testing the effectiveness of the sample preparation using the optimized design. These two phases will be explained in detail in the following sections.

Design optimization experiments

Initially, the effectiveness of pouring is studied to minimize residual liquid after pouring due to surface tension. In order to quantify the effectiveness of pouring, the mass of the tube is measured before loading the sample, after loading the sample, and after pouring out the sample. The sample used for this test is 900 μl of water. The tube is then placed in the device which pours the tube's contents by rotating the tube motor. After returning to the home position, the mass of the tube is measured again. The difference between this value and the previously measured mass indicates the amount of residual water that is not effectively removed from the tube by the pouring process due to surface tension. This experiment is performed using conical-bottomed and round-bottomed tubes to study the effect of the tube's shape on supernatant removal.

The effectiveness of the automated magnetic bead separation is also studied for the optimization of the device. Because the magnetic beads retain DNA within the sample tube while the supernatant is being removed, the effective retention of the magnetic beads translates to effective isolation of DNA from the sample. To perform this experiment, a tube containing 200 μl of suspended magnetic bead mixture is placed in the device with the magnet in the upward position ($\theta_1 = 180°$). Next, the device's magnet is moved to the downward position ($\theta_1 = 0°$) using the magnet motor, and the magnetic beads are attracted toward the tube's wall for 30 seconds. After 30 seconds, the tube motor and magnet motor simultaneously rotate to position the sample tube and the magnet for pouring. The supernatant is poured into a separate waste tube. The device then returns the tube to the home position and the magnet to the upward position. It then resuspends the magnetic beads in 200 μl of water by operating the peristaltic pump.

Sample preparation experiments

In a laboratory setting, the gold standard sample preparation methods are magnetic bead-based DNA separation and silica filter-based DNA extraction. The automated DNA extraction using the proposed device is compared with the two gold standard methods for DNA isolation yield. Each of these methods rely on whole human blood which is spiked with foreign DNA. For testing purposes, spiked whole blood with

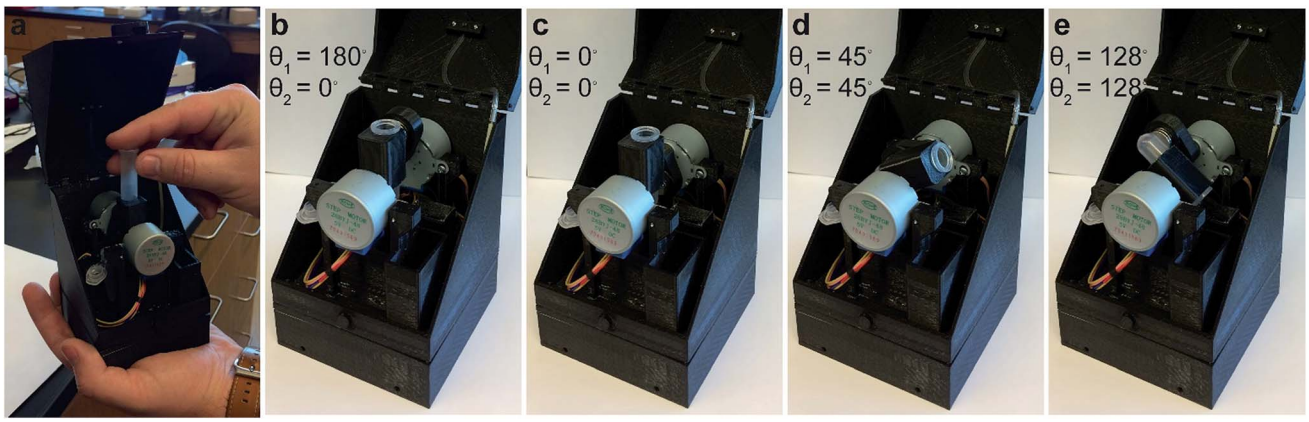

Fig. 4 The automated handheld device which can perform magnetic bead-based sample preparation using two simple θ motions. (a) The insertion of the sample into the device, (b) the initial (home) position, (c) the engagement of magnet ($\theta_1 = 0°$) to start the magnetic bead separation, (d) simultaneous rotation of both motors (θ_1 and θ_2) to initiate pouring, and (e) the pouring position ($\theta_1 = \theta_2 = 128°$).

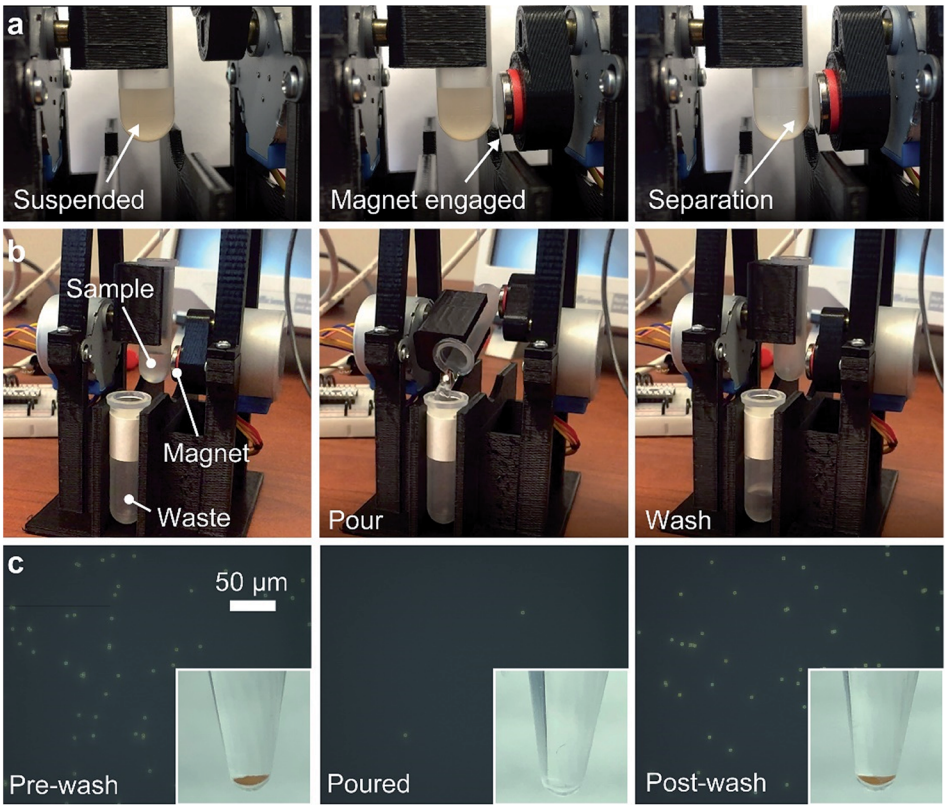

Fig. 5 The photographs of the magnetic bead separation and pouring method. (a) The suspended magnetic beads in the tube are attracted toward the magnet once the magnet engages into position. This results in an accumulation of magnetic beads on the tube's wall. (b) To remove the supernatant liquid for washing steps, both motors for the tube and magnet simultaneously rotate. (c) The microphotographs of pre-wash, poured, and post-wash liquids to show an effective retention of magnetic beads in the tube after washing and pouring.

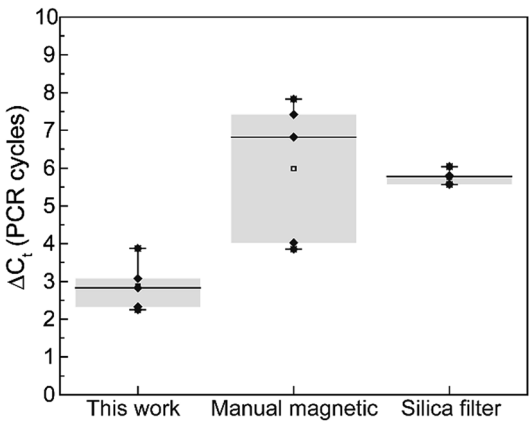

Fig. 6 The comparison of DNA capture yields between automated, manual magnetic bead-based separation, and silica filter-based DNA extraction ($n = 5$ for each method). Deviations (ΔC_t) from the positive control is 2.872 ± 0.262 (SEM), 5.988 ± 0.764 (SEM), and 5.756 ± 0.077 (SEM). The low ΔC_t level of the automated method indicates an efficient DNA capture compared to that of gold standard methods.

mouse DNA containing *SOX2* gene is used to reduce the risk of infection to the researchers.

Methods relying on magnetic bead DNA isolation protocol, both automated DNA extraction as well as manual DNA extraction, are carried out as described by the DynaBeads DNA DIRECT Blood user guide. Initially, 100 μl of DNA-spiked whole human blood is placed into a 2 ml round-bottomed tube. 1 ml of chilled (−20 °C) Red Cell Lysis Buffer is added to the tube and the solution is allowed 5 minutes for lysis to complete at room temperature. The tube is occasionally agitated for thorough lysis. Once lysed, the tube is centrifuged at 8000 RPM for 2 minutes which forms a pellet on the bottom of the tube. The supernatant is then carefully removed and 200 μl of well-mixed magnetic bead suspension is added. This mixture is now allowed to incubate at room temperature for 5 minutes, allowing DNA to attach to the beads. The protocol then diverges between the manual and automated methods.

For the manual method, the tube is placed on a holder containing the same magnets used in the machine. The tube is allowed to sit for one minute allowing the magnet to attract the beads to the wall of the tube. After one minute, the supernatant is carefully pipetted out by hand and discarded. Next, 1 ml of washing buffer is quickly pipetted into the tube in a way that washes the magnetic beads off the tube wall. The tube is then placed back onto the magnet and the process is repeated two times, giving a total of three washing–separating–resuspending cycles. After this, the beads are again pulled to the wall and the supernatant is discarded.

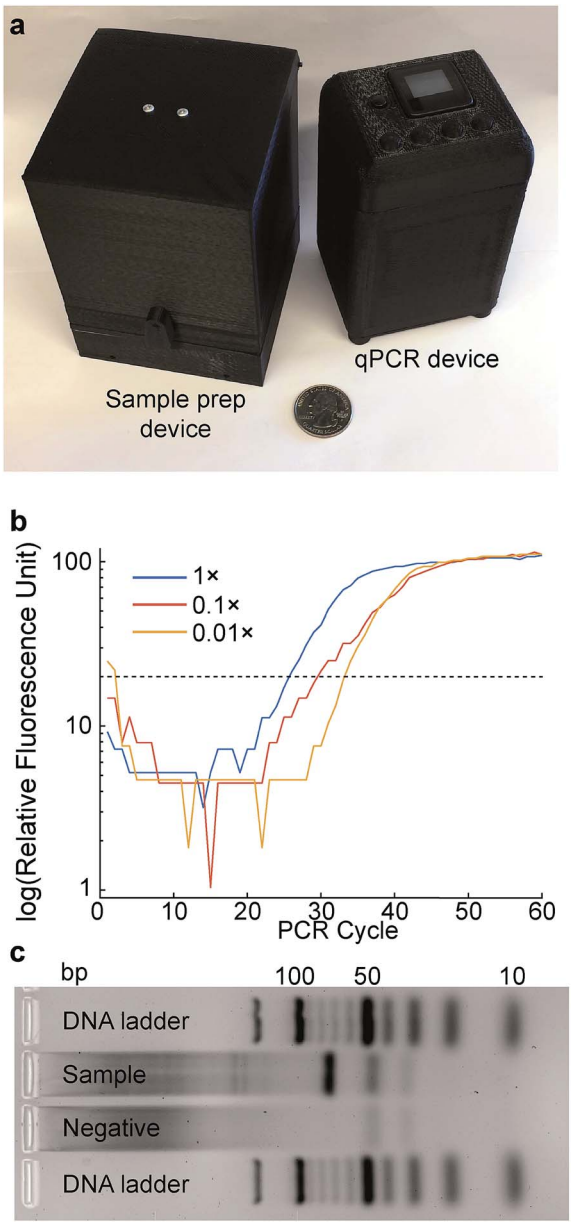

Fig. 7 (a) A comprehensive, portable, battery-operated diagnostic system using the automated sample preparation device and 3D-manufactured qPCR device. (b) Fluorescence reading, in logarithmic scale, from the handheld qPCR device for three different concentrations of target DNA spiked in whole blood. For 1×, 0.1×, and 0.01× samples, C_t values are 24.90, 28.57, and 32.33, respectively. (c) Gel electrophoresis of the qPCR product, confirming a 68 bp band.

For the automated method using our sample preparation device, the device is first prepared by adding 3 ml of washing buffer to the reservoir. The sample tube is then placed into the device which is subsequently turned on. Once activated, the device brings the magnet to the upward position and simultaneously begins swinging the sample tube so that gentle mixing is performed for one minute. Next the tube is brought to home position and the magnet is brought to the downward position, allowing the magnetic beads to move to the tube wall. After 30 seconds, the magnet and tube rotate to discard the supernatant by pouring. The tube then returns to the home position, the magnet to the upward position, and the peristaltic pump is activated to pump 900 μl of washing buffer into the tube. After resuspension, the cycle repeats for a total of three washing–separating–resuspending cycles. The final resuspension does not take place, and the device simply returns to the home position, allowing for the user to easily remove the sample tube, which at this point contains only magnetic beads still attached to the wall.

For both the manual and the automated magnetic bead protocols, the user adds 200 μl of resuspension buffer to the tube to wash the beads from the wall. The sample tubes are incubated at 65 °C for 5 minutes, this incubation elutes the DNA from the magnetic beads. After elution, the tubes are placed on the magnet for 1 minute to pull the beads to the tube wall. The supernatant is then collected and transferred to new clean PCR tubes for qPCR quantifications. For each experiment, the C_t of the captured DNA sample is subtracted from the C_t measured from the positive control, to result in ΔC_t. ΔC_t reveals the ratio of DNA between the positive control and the captured DNA from each respective method.

For the silica filter-based isolation protocol, the process is conducted based on the QIAamp DNA Blood Mini Kit user guide. The first step to carry out this process is to pipette 20 μl of protease K in a 1.5 ml tube. The use of the protease is necessary in order to breakdown proteins in 200 μl of the sample, DNA spiked-human blood. Also, 200 μl of lysis buffer is added. The tube is agitated to thoroughly allow efficient lysis before incubating it for ten minutes at 56 °C and centrifuging to remove the evaporated drops. Next, to enhance the binding of the DNA to the silica, 200 μl of ethanol is mixed in. Afterward, the solution is pipetted into a spin column to centrifuge for one minute at 8000 RPM. During this step, DNA binding to silica occurs. The process repeats with 500 μl of washing buffer, which is centrifuged at 8000 RPM for one min, followed by another 500 μl of washing buffer, which is centrifuged at 14 000 RPM for three minutes. After each time a spin column is used and centrifuged, the collection tube is discarded and replaced with a new one. Multiple washes ensure that the DNA is purified. After discarding the collection tube, the tube is then centrifuged again for one minute to guarantee complete liquid removal. After transferring the spin column into a 1.5 ml tube, 200 μl of elution buffer is added before centrifuging one more time. This step weakens the binding of DNA to the silica filter and elutes DNA into the elution buffer.

PCR experiments

Each experiment proving the validity of the sample preparation device are run using qPCR. Three sets of experiments: manual magnetic bead method, automated magnetic bead method, and silica filter method, are run using a conventional qPCR machine (7900HT Fast Real-Time PCR System, Applied Biosystems, Foster City, CA). PCR reagents are from VitaNavi Technology (Ballwin, MO) with a TaqMan probe that targets mouse *SOX2* gene from Applied Biosystems (Waltham, Massachusetts).

Target DNA was sourced from ABM (Vancouver, Canada). Human blood was supplied by Zen Bio (Research Triangle Park, NC). The DynaBeads DNA direct blood kit by Invitrogen (Carlsbad, CA) was used for magnetic bead experiments. For silica bead experiments, the QIAamp system by Qiagen (Hilden, Germany) was used. The presented device is also tested in conjunction with a 3D-manufactured qPCR device described previously.[14] For PCR reactions, each well, or tube (for reactions ran with the 3D-manufactured qPCR machine) are prepared as follows: 6 μl of PCR grade water, 10 μl of PCR mix, 1 μl of polymerase, 1 μl of TaqMan, and 2 μl of the desired sample. For PCR reactions performed in the 3D-manufactured qPCR device, an additional 20 μl of mineral oil is added to prevent evaporation of the reagents during thermocycling. The product length of the qPCR is measured by using a gel electrophoresis. A 3% agarose gel with a channel length of 100 mm is ran at 75 V for 2 hours (50 ml of 1× TBE, 1500 mg agarose powder and 5 μL of 10 000× SYBR safe gel stain).

Results and discussion

The comparison of pouring effectiveness between conical-bottomed tubes and round-bottomed tubes is shown in Fig. 3. Rotating the tube motor by 128° results in a reliable pouring of bulk fluid from the bottom of the tube (Fig. 3a). Steeper angles do not achieve any observable improvements in fluid removal. The residual liquid is quantified by subtracting the weight of the tube before (w_{water}) and after (w_{poured}) pouring. In Fig. 3b, the effectiveness of pouring is plotted in percentile using eqn (1).

$$\text{Pouring } (\%) = \frac{w_{\text{water}} - w_{\text{poured}}}{w_{\text{water}} - w_{\text{tube}}} \quad (1)$$

With conical-bottomed sample tubes, the pouring method yields 85.7% (with the standard error of the mean (SEM) ± 0.67%) removal of water and the use of round-bottomed sample tubes yields effective removal of 99.52% (with SEM ± 0.19%). Each experiment is repeated for 10 times ($n = 10$). The retention of water in the conical-bottomed tube is due to the water's adhesive force in the narrow conical bottom, supporting the weight of water in the presence of gravity. The round bottom forms much less overall adhesive force, resulting in an effective removal of water in the tube by simple pouring (Fig. 3c).

The automated sample preparation device is constructed with the pouring scheme to perform the magnetic bead-based separation (Fig. 4). The handheld device is completely portable, and battery operated. The internal batteries (3000 mA h) have enough capacity to power the stepper motors continuously for 13 hours, giving ample battery life for many sample preparations on a single charge. The device is operated by inserting the sample in a round-bottomed tube (Fig. 4a). The device's power is turned on by sliding the power switch (Fig. 2b). Initially, the motors are in a home position ($\theta_1 = 180°$, $\theta_2 = 0°$, Fig. 4b). By rotating the magnet motor ($\theta_1 = 0°$), magnetic separation can be started (Fig. 4c). For pouring, both motors turn to 128° (Fig. 4d and e). The combination of motions tested above can perform the sample preparation autonomously.

The effectiveness of the separation and pouring is tested. To visualize the functionality of the device, a copy of the device without the enclosure is used (Fig. 5). A sample round-bottomed tube with 200 μl of magnetic beads is inserted into the test device. After the magnet engages near the tube, the suspended beads are separated and accumulated on the tube's wall (Fig. 5a). Afterwards, the magnet and tube motors rotate to 128° to pour the supernatant liquid (Fig. 5b). The washing step is performed by dispensing washing buffer into the tube, which causes a resuspension of the magnetic beads. The original (pre-wash) sample, the supernatant (poured) sample, and the resuspended (post-wash) sample are inspected under a microscope (Fig. 5c). As expected, the poured sample contains few magnetic beads and the post-wash sample contains a comparable amount of beads to the pre-wash sample. This experiment demonstrates a successful pouring, removing the supernatant liquid without significantly disturbing the separated beads.

The DNA capture efficiencies of the developed device, manual magnetic bead-based separation, and silica filter-based DNA extraction are compared (Fig. 6). For each of the extraction methods, qPCR is performed to determine the ΔC_t, which can be correlated to the ratio of concentrations between the original DNA samples before the extraction and the extracted DNA samples. The automated method on average resulted in a deviation from the positive control by 2.872 cycles with a SEM of 0.262 cycles, the manual magnetic bead experiments deviated by 5.988 cycles with a SEM of 0.764 cycles, and the silica filter method deviated by 5.756 cycles with a SEM of 0.077 cycles ($n = 5$ for each method). Each ΔC_t can be interpreted as 13.7%, 1.58%, and 1.85% for automated, manual, and silica-filter-based methods, respectively. These yields are low considering that each PCR cycle signifies a 2× amplification. However, due to the short length of the target used in this experiment (68 bp) this is expected as these DNA purification techniques are generally not optimized for such short DNA fragments, which is consistent with previously reports of less than 10% recovery for DNAs with shorter than 75 bp.[15–17] Consequently, the most important metric by which to evaluate the effectiveness of the automated method is in comparing it directly with other methods that use the same target. This data suggests that the automatic sample preparation method has a higher DNA capture yield than performing the same operation manually or by using an alternative method such as a silica filter for this length of DNA. This study does not guarantee an increased yield of any DNA length. However, it is likely to result in a comparable yield to the manual magnetic bead-based separation because the fundamental mechanism for capturing DNA is identical. In Fig. 6, each bar represents data from each of the methods. The increased yield (lower deviation from the positive control) of the automated method is clearly evident. One possible explanation for the increased yield is the effectiveness of the pouring motion for supernatant removal and reduced risk of disturbing the bead cluster while removing the supernatant liquid, an advantage that is unique to this device. Additionally, the pouring motion allows for a contactless sample preparation protocol

with fewer components requiring replacement between samples. The contactless nature of the device also reduces the risk of contamination compared to other devices due to the reduced number of possibly contaminated components that come in contact with the sample. Although the sample is exposed to the air, it has been found that air is rarely the source of PCR contaminants.[18] In order to determine the lowest number of DNA copies the presented device is capable of capturing, a blood sample containing different number of DNA copies (9.6×10^5, 9.6×10^4, and 9.6×10^3) is processed using the automated sample preparation device and detected using the conventional qPCR. The average C_t for each sample is 28.07 ± 0.54, 33.00 ± 0.14, and 33.90 ± 0.16, respectively. Between 9.6×10^5 and 9.6×10^4 samples, the C_t values are significantly different, however, reducing the sample below 9.6×10^4 did not result in a noticeable change. Thus, the extraction limit is on the order of 10^4 DNA copies.

The novelty of the system is to be able to operate complicated sample preparation tasks outside of a laboratory setting where tools, such as pipettes, are not readily available. We have previously reported a 3D-manufactured qPCR device which is also battery operated and a handheld device.[14] The combination of two presented devices (Fig. 7a) creates a unique opportunity in point-of-care (POC) diagnostics by minimizing the user's involvement in performing a high-quality qPCR-based diagnosis of infectious diseases. To test this, samples are prepared using the automated magnetic bead sample preparation device and analyzed with the 3D-manufactured qPCR device from a previous study.[14] This experiment begins with a concentrated DNA sample (1×) that is diluted 10 times (0.1×) and diluted 10 times again (0.01×). The concentrated and two diluted DNA samples are used to spike 100 μl of blood used in the experiments. The qPCR measurements from the 3D-manufactured device are shown in (Fig. 7b). C_t values of the 1×, 0.1×, and 0.01× samples are 24.90, 28.57, and 32.33, respectively. Each dilution is separated by 3.67 and 3.76 cycles, which closely matches the ideal separation of 3.33 cycles between 10× dilutions. To confirm the amplification of the correct target DNA, a gel electrophoresis is performed (Fig. 7c). The result shows a band near 70 bp indicating the amplification of target gene. The byproducts of TaqMan probe are also visible as expected.[14,19] This experiment reveals the devices' robust operation and consistent DNA extraction as well as a portable handheld system that comprehensively performs qPCR measurements. qPCR allows for not only a positive/negative diagnostic result, but a quantitative one as well. This will aid in determining the proper treatment when used to diagnose an infection.

Conclusion

This work has demonstrated the effectiveness of an automated sample preparation device that is capable of extracting DNA used for PCR reactions from whole blood samples. In fact, the device has been proven to result in a higher DNA yield than other common methods, which will result in a lower minimum detectable level of infection using qPCR. It has also shown the effectiveness of the isolated DNA sample when used with the authors' portable real-time qPCR machine. The work demonstrated a crucial step toward producing an autonomous nucleic acid testing method using qPCR, a gold standard test for many infectious disease diagnoses. However, a few of the steps, such as adding the magnetic beads and lysis buffer with the blood into a tube as well as adding qPCR reagents into the purified DNA, still require user's handling. For future work, we are actively working toward integrating every step involved in qPCR-based diagnostics. For mixing magnetic beads and lysis buffer, two options are possible: (1) a pre-loaded tube with beads and lysis buffer, and (2) additional dispensers in the device. Additionally, qPCR reagents have a short shelf life at room temperature and thus cannot reliably be stored in liquid form. Lyophilization can be used to aid in storage, and is proven to elongate the shelf life of chemicals and drugs.[20]

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgements

The authors thank the graduate research assistantship support from the Office of Research and Commercialization (ORC) at the University of Central Florida (UCF) for an ORC Doctoral Fellowship. We also acknowledge the partial support from the Office of Undergraduate Research (OUR) at UCF for an OUR Student Research Grant. Human whole blood was purchased from Zen-Bio Inc.

Notes and references

1 S. P. Johnston, N. J. Pieniazek, M. V. Xayavong, S. B. Slemenda, P. P. Wilkins and A. J. da Silva, *J. Clin. Microbiol.*, 2006, **44**, 1087–1089.
2 A. St John and C. P. Price, *Clin. Biochem. Rev.*, 2014, **35**, 155–167.
3 R. Boom, C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen and J. van der Noordaa, *J. Clin. Microbiol.*, 1990, **28**, 495–503.
4 R. Marshall, M. Chernesky, D. Jang, E. W. Hook, C. P. Cartwright, B. Howell-Adams, S. Ho, J. Welk, J. Lai-Zhang, J. Brashear, B. Diedrich, K. Otis, E. Webb, J. Robinson and H. Yu, *J. Clin. Microbiol.*, 2007, **45**, 747–751.
5 K. M. Koo, E. J. H. Wee, Y. Wang and M. Trau, *Lab Chip*, 2017, **17**, 3200–3220.
6 J. A. DuVall, D. Le Roux, A.-C. Tsuei, B. L. Thompson, C. Birch, J. Li, D. A. Nelson, D. L. Mills, M. M. Ewing, R. S. McLaren, D. R. Storts, B. E. Root and J. P. Landers, *Anal. Methods*, 2016, **8**, 7331–7340.
7 W. Zhang, S. Guo, W. S. Pereira Carvalho, Y. Jiang and M. J. Serpe, *Anal. Methods*, 2016, **8**, 7847–7867.
8 S. Byrnes, A. Fan, J. Trueb, F. Jareczek, M. Mazzochette, A. Sharon, A. F. Sauer-Budge and C. M. Klapperich, *Anal. Methods*, 2013, **5**, 3177–3184.

9 J. R. Buser, X. Zhang, S. A. Byrnes, P. D. Ladd, E. K. Heiniger, M. D. Wheeler, J. D. Bishop, J. A. Englund, B. Lutz, B. H. Weigl and P. Yager, *Anal. Methods*, 2016, **8**, 2880–2886.
10 T. Baier, T. E. Hansen-Hagge, R. Gransee, A. Crombé, S. Schmahl, C. Paulus, K. S. Drese, H. Keegan, C. Martin, J. J. O'Leary, L. Furuberg, L. Solli, P. Grønn, I. M. Falang, A. Karlgård, A. Gulliksen and F. Karlsen, *Lab Chip*, 2009, **9**, 3399.
11 J. Siegrist, R. Gorkin, M. Bastien, G. Stewart, R. Peytavi, H. Kido, M. Bergeron and M. Madou, *Lab Chip*, 2010, **10**, 363–371.
12 Q. Ramadan and M. A. M. Gijs, *Analyst*, 2011, **136**, 1157.
13 R. S. Sista, A. E. Eckhardt, V. Srinivasan, M. G. Pollack, S. Palanki and V. K. Pamula, *Lab Chip*, 2008, **8**, 2188–2196.
14 G. Mulberry, K. A. White, M. Vaidya, K. Sugaya and B. N. Kim, *PLoS One*, 2017, **12**, 1–18.
15 I. Glocke and M. Meyer, *Genome Res.*, 2017, **27**, 1230–1237.
16 F. Santos, S. Gómez-Manzo, E. Sierra-Palacios, A. González-Valdez, A. Castillo-Villanueva, H. Reyes-Vivas and J. Marcial-Quino, *MethodsX*, 2017, **4**, 289–296.
17 R. Zhang, K. Nakahira, X. Guo, A. M. K. Choi and Z. Gu, *Sci. Rep.*, 2016, **6**, 36097.
18 N. Witt, G. Rodger, J. Vandesompele, V. Benes, A. Zumla, G. A. Rook and J. F. Huggett, *J. Biomol. Tech.*, 2009, **20**, 236–240.
19 S. Troxler, A. Marek, I. Prokofieva, I. Bilic and M. Hess, *J. Clin. Microbiol.*, 2011, **49**, 1339–1346.
20 P. Yadava, M. Gibbs, C. Castro and J. A. Hughes, *AAPS PharmSciTech*, 2008, **9**, 335–341.