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ORIGINAL PAPER

Paper-based device with on-chip reagent storage for rapid extraction of DNA from biological samples

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Abstract Conventional methods for extraction of DNA are expensive, time-consuming and tedious. To overcome these limitations, a paper-based DNA extraction device was developed that incorporates sponge-based buffer storage, a paper-based valve and channels of different length to autonomously direct the reagent and sample to the Fusion 5 disk for DNA capturing. With this device, DNA can be extracted within 2 min from only 30 μL samples of whole blood, serum, breast cancer cell, saliva, sputum and bacterial suspension. The device can also extract Hepatitis B Virus DNA from clinical blood samples and after quantification shows a detection limit as low as 10^4 copies·mL^{-1}. This highlights its potential use in future diagnostics. The performance of the device was similar to that of the commercial QIAamp DNA micro kits and the FTA card. In our perception, this simple, inexpensive, portable and disposable device holds great promise in terms of POC testing in resource-poor settings.

Keywords Nucleic acid extraction · Whole blood · Serum · MCF7 cell · Saliva · Sputum · Bacteria · Point-of-care testing

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Introduction

Deoxyribonucleic acid (DNA) is often used as important biomarkers of organism for disease diagnosis [1, 2], environment monitoring [3] and food safety detection [4], especially in resource limited settings. Thus, the rapid and accurate detection of DNA targets in various biological samples are needed [5–8]. However, DNA is usually located in complex biological samples (e.g., whole blood, saliva, urine, bacteria), making it difficult to be analyzed directly. Therefore, DNA extraction is essential for downstream analysis. Conventionally, various methods have been developed for DNA extraction, such as phenol-chloroform method [9], spin-column based method [10] and magnetic-bead based method [11]. However, these methods are high-cost, time-consuming, complex and labor intensive, which limit their use at point of care (POC). Therefore, there is an urgent need for a low-cost, rapid, simple, portable and disposable nucleic acid extraction device.

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Paper has become increasingly attractive for the development of paper-based analytical devices for various diagnostics applications [12–14]. Nowadays, paper has also been used to fabricate DNA extraction devices for use in POC settings [2, 15]. For example, filtration isolation of nucleic acid (FINA) method has been developed for extraction of Human Immunodeficiency Virus Type 1 (HIV-1) proviral DNA from whole blood using a single layer matrix membrane disk (Fusion 5) within 2 min [16]. However, the device does not allow reagent storage and requires manual addition of reagent onto the paper disk. Additionally, an extraction device integrating paper and plastic into microfluidic device has been developed to extract DNA from diverse biological samples within 7 min [17]. However, this device needs extra syringe pumps and valves, and does not allow on-chip reagent storage either. To date, several paper-based studies and commercial products have been solved the problem of reagent storage. For instance, a paper-based origami device with dry reagent storage has been developed for DNA extraction from mucin and sputum [18]. Similarly, commercial filter papers (e.g., FTA elute card [15] and FTA classic card [2]) with dry reagent storage have been used to extract DNA from different biological samples (e.g., whole blood, bacteria, tissue, cervical cells [16, 19]) prior to amplification and detection. Although dry reagent storage on chip has been achieved, these methods need ultrapure water to dissolve the stored dry reagents, which involves multiple washing steps and is time-consuming (1.5 ~ 2 h to complete the extraction process). Thus, there is an unmet demand for rapid and simple DNA extraction device with liquid reagent storage. Besides, the aforementioned DNA extraction methods require an extra timer to track the end point of the assay. As an alternative, paper-based fluidic timer has been introduced to track the assay time, avoiding the need for batteries or electrical powers [20]. Therefore, integrating the cost-effective timer into the paper-based analytical device holds great potential to achieve a rapid DNA extraction at POC. To the best of our knowledge, even though many paper-based extraction devices have been reported [16, 18, 21], the development of a simple, cost-effective, rapid paper-based extraction device with reagent storage property, which is compatible with POC application has not yet been demonstrated.

Herein, we developed a paper-based device for DNA extraction from biological samples by integrating sponge-based reservoir module and paper-based valve into one single paper-based device. The paper-based DNA device can realize one-step DNA extraction from diverse biological samples (Fig. 1), such as whole blood, serum, MCF7 cell, saliva, sputum and bacteria. In addition, the performance of DNA extraction in clinical sample testing (hepatitis B virus (HBV) as model analyte) using our device was comparable to that of commercial QIAamp® DNA Micro kits and FTA card. We envision that our prototype can be broadly applied to various target analytes, offering a great potential for point-of-care testing (POCT) in resource-poor settings.

Experimental method

Fabrication of paper-based DNA extraction device

The device was designed by using Solidwork and then printed by 3D–printer using photopolymer resin (Formlabs Co., Ltd., USA, formlabs.com) (Fig. 1a), which was composed of the top cover (Fig. 1b) and the integrated platform (Fig. 1c). The top cover included button, inlet hole, outlet hole and timer hole (Fig. 1b). The button was fixed on the top cover by bridge (**Fig. S1A**). The integrated platform consisted of a reservoir module and a paper-based module (Fig. 1d) supported by a substrate (Fig. 1e). The reservoir module (**Fig. S1B**) included sponge (washing buffer reservoir (diameter: 10 mm, height: 5 mm), lysis buffer reservoir (diameter: 8 mm, height: 5 mm) and timer buffer (diameter: 8 mm, height: 5 mm), rubber seal (the size was the same with the sponge) and small exit (2 mm × 5 mm) (**Fig. S1C**). The paper-based module (Fig. 1d) was composed of three channels (first-paper-based time channel (35 mm × 5 mm), second-lysis channel (25 mm × 5 mm), third-washing channel (10 mm × 5 mm), absorption pad (18 mm × 20 mm) and time zone (diameter: 4 mm). The timer zone was connected with first channel. The 7 mm × 5 mm of sample area filter (the distance between the small exit and sample area was 15 mm), a small piece of filter paper connected to the sample area, Fusion 5 disk of DNA capture area (diameter: 3 mm) and absorption pad were placed on second channel. The channel was composed of paper-based valve, which was composed of three layers fold paper and showed "ㄱ "shape (Fig. 1d).

DNA isolation

DNA was extracted from various biological samples using the paper-based DNA extraction device. The DNA extraction buffer was prepared according to the published protocol [16] with slight modification. Washing buffer (50 mM NaOH), lysis buffer (5% Triton-X 100) and time buffer (PEG8000 buffer) were respectively stored in three separate sponges with the diameters of 10 mm, 8 mm and 8 mm, which were placed into the reservoir module. The entire process was briefly discussed in the discussion (Fig. 2).

Optimization of paper-based DNA extraction device

(Please see the details in the supplementary information)

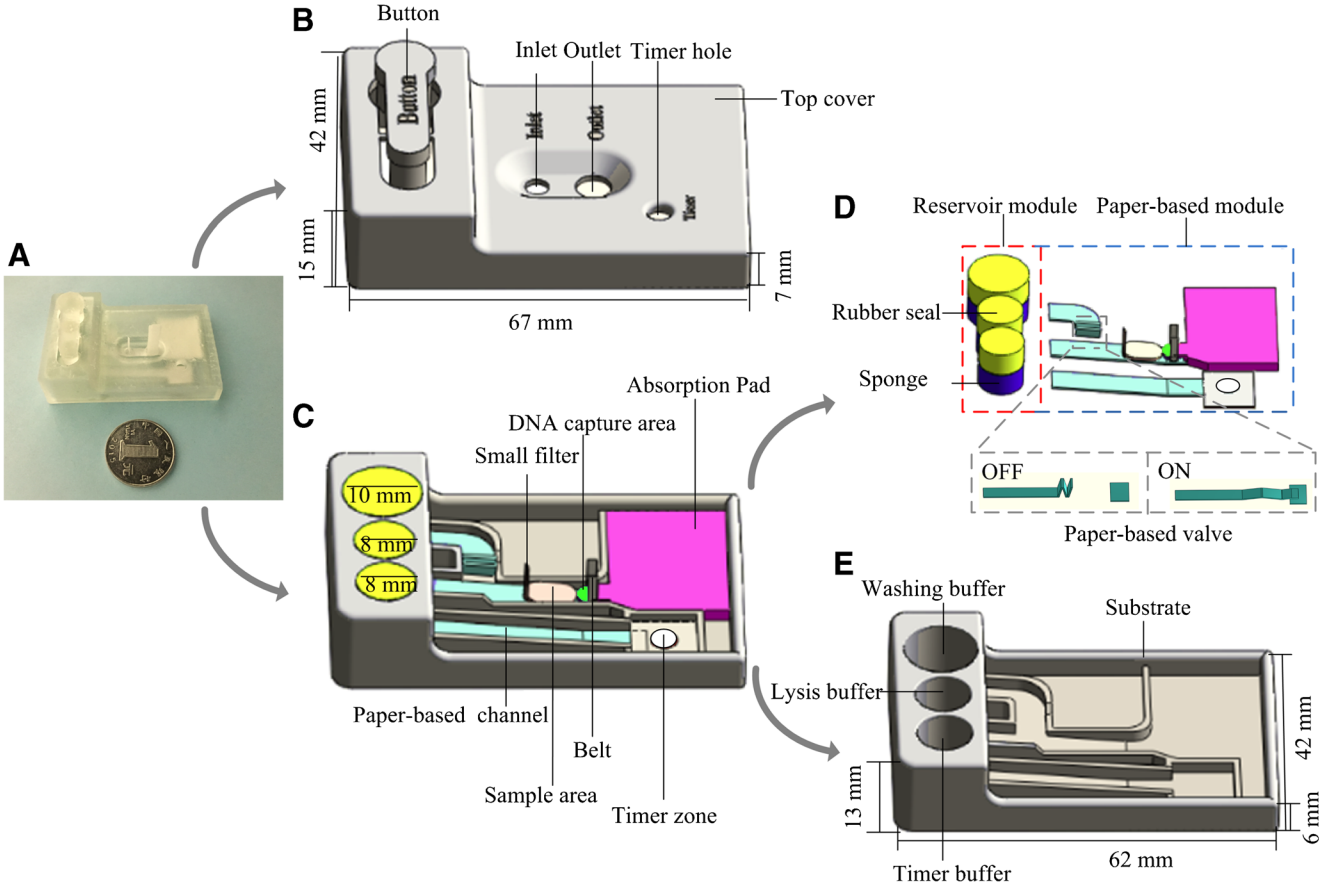

Fig. 1 Schematic of the paper-based DNA extraction device. **a** The photo image of paper-based DNA extraction device; **b** The top cover of the paper-based device; **c** The integrated platform of reservoir module and paper-based module supported by substrate; **d** The structure of reservoir module and paper-based module (including the paper-based valve); **e** The substrate

DNA extraction from various biological samples

Human whole blood, human serum, human MCF7 cells, human saliva and human sputum and Escherichia coli (*E. coli*) suspension (ATCC 25922) were used as diluent for target DNA, which were extracted using commercial QIAamp® DNA Micro kit, FTA card and the paper-based DNA extraction device, respectively. By using QIAamp® DNA Micro kit, 30 μL of sample was used for DNA extraction, and 10 μL of elution buffer was used to elute the DNA for the downstream process (PCR/qPCR). By using FTA card, 30 μL of sample was added on FTA card (with diameter of 3 mm). After washing, the DNA was captured by the FTA card, which was used for the downstream process. By using paper-based DNA extraction device, 30 μL of sample was used for DNA extraction. Then, the DNA was captured by Fusion 5 disk, which was used for the downstream process.

DNA detection

After the extraction, PCR and qPCR were performed with the use of β-actin primers [22] and the *E. coli* primers [6] using ABI veriti 96 well thermal cycler (ABI, USA, www.abiresearch.com) and ABI 7500 Fast Real-time PCR system (ABI, USA, www. abiresearch.com). qPCR was used to detect the efficiency of extraction and performed based on Trans Start® Tip Green qPCR SuperMix assay with 30 μL reaction volume. The primers for the human β-actin gene were synthesized based on a previously published protocol [22]. The PCR products were analyzed by electrophoresis on 2% EB-stained agarose gels in Tris acetate-EDTA (TAE) buffer. The gel images were captured and analyzed using a ChemiDoc MP imaging system (Bio-Rad, USA, www.bio-rad.com). qPCR was evaluated by CT values.

Comparison of the limit of detection of three extraction methods

Firstly, repeated freezing and thawing method was used to prepare different negative samples, and then PCR was used to prove the absence of specific amplification band. First, beta actin DNA from human MCF7 cells and *E. coli* DNA were extracted by phenol chloroform method with the concentration determined by Nanophotometer (Implen, Germany,

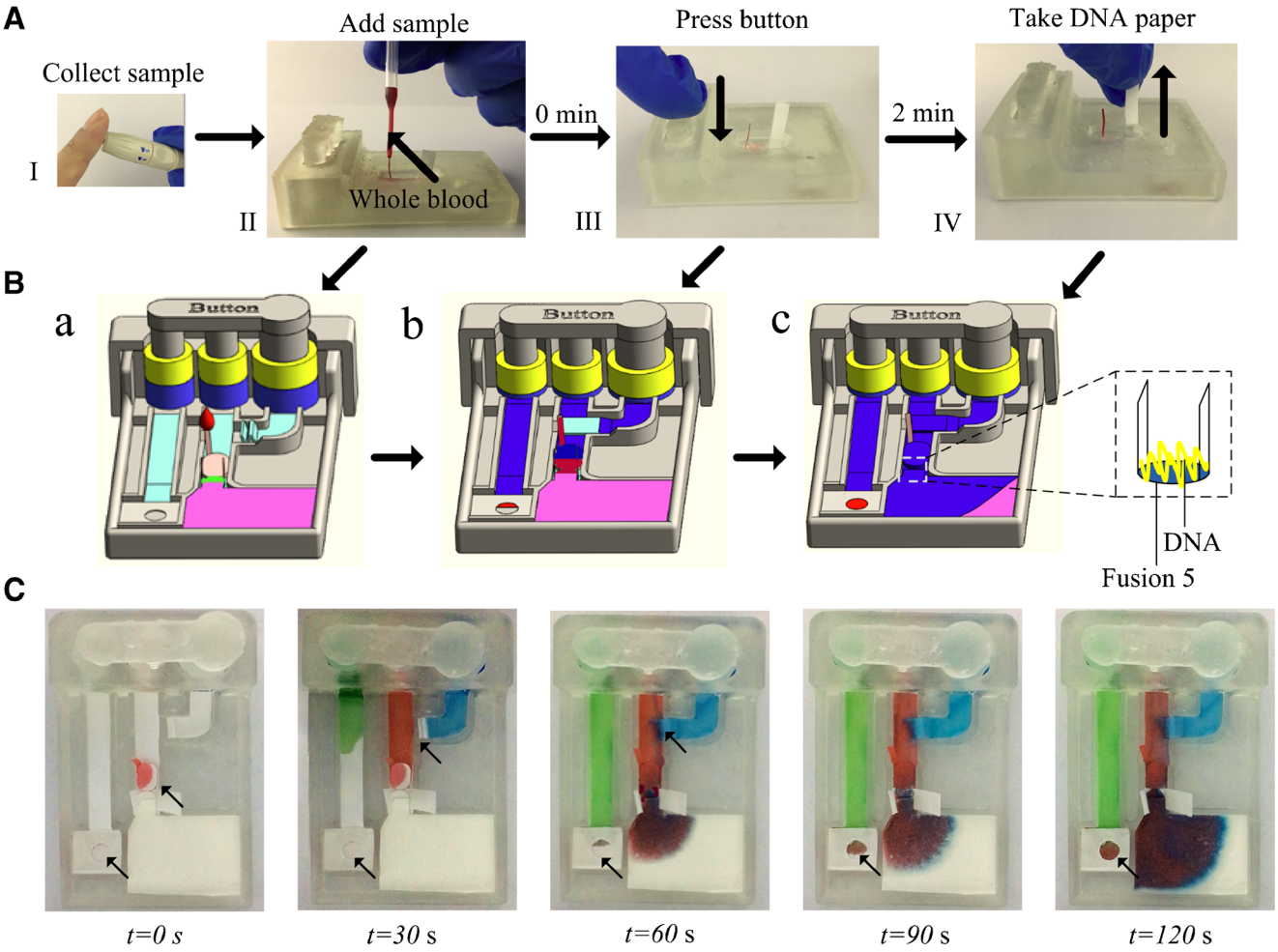

Fig. 2 The process of DNA extraction using the paper-based DNA extraction device. **a** A schematic diagram of DNA extraction. (**I** The fingertip was pricked using a lancing device; **II** A drop of blood was then applied to a small piece of filter paper connected to the sample area; **III** The start button was pressed to start the DNA extraction process; **IV** Collection of Fusion 5 disk.). **b–c** The flow sequence in the extraction device, starting from the flow of lysis buffer to lyse sample, at the same time, the flow of washing buffer to activate valve and purified the DNA, and finally the color changed in timer zone, and the collection of Fusion 5 disk for downstream analysis

www.implen.de). Then, different amount of beta actin DNA or *E. coli* DNA (10^3, 10^2, 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} ng) were spiked into 30 μL of different negative samples and 0.01 M pH 7.4 PBS buffer (as negative control). Following the extraction and amplification by PCR, the results were determined by 2% agarose gel, then the optical density of bands were analyzed by ImageJ 6.0.

The recovery rate of paper-based DNA extraction device

A quantification standard curve was prepared by performing the serial dilutions of β-actin DNA of human MCF7 cells (from 5 ng to 640 ng per reaction) using qPCR. The recovery rates of β-actin DNA extraction using three DNA extraction methods were evaluated by quantifying the β-actin concentration through qPCR from different spiked biological samples. The amount of DNA in control sample was determined and the recovery rate (%) was calculated by determining the ratio of β-actin amount from each sample over the control.

Preparation of external standard of real time PCR detection assay

Additionally, the positive HBV serum was prepared in serial dilutions from 10^7 to 10^0 copies·mL^{-1} to create a standard curve. 30 μL of serum was utilized for DNA extraction. Then, the template was used for qPCR. CT values were plotted against log (copy number) by ABI 7500 Fast Real-time PCR system software to obtain a standard curve. Slope parameters were estimated by linear regression of CT values vs. log (copy number). For the slope estimates, 95% Confidence Intervals were calculated using OriginPro 8.0 software. The slope value was applied to calculate the PCR efficiency.

Extraction and detection of clinical blood samples

Clinical samples were supplied by the First Affiliated Hospital of Xi'an Jiaotong University. The study was approved by the Institute Research Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. Blood samples were collected from 12 patients with clinically confirmed HBV infection and quantified using qPCR. The data of paper-based device were compared with that of commercial QIAamp® DNA Micro kits and FTA cards. The primers were selected according to a previously published protocol [23].

Results and discussion

Paper-based DNA extraction device design

In DNA analysis, conventional DNA extraction methods are time-consuming, equipment-dependent and involve multiple-step operations, restricting their application in POC settings. To overcome these drawbacks, a low-cost, rapid, portable and disposable paper-based DNA extraction device was developed to achieve one-step DNA extraction (Fig. 1a). The device was consisted of a top cover (Fig. 1b) which was initially connected to start button by the bridge (**Fig. S1A**) before the extraction begins, and an integrated platform (Fig. 1c) containing both reservoir module and paper-based module (Fig. 1d) supported by a substrate (Fig. 1e). In the reservoir module, three sponges were used to store the reagents, and a rubber seal was placed on top of the sponge (**Fig. S1B**) to prevent reagent evaporation. To start the extraction process, the start button was pressed, and then the rubber seal and the sponge were compressed to allow the stored buffers to flow through the paper by small exit. After the compression, the shape of the sponge would be maintained in the compressed form due to the presence of static friction between the rubber seal and the reservoir wall (**Fig. S1C**, **Supplementary Movie S1**).

The paper-based module was consisted of time module and extraction module. The time module included 6% PEG solution, paper-based channel, and the label paper. 6% PEG solution was extruded and then flowed through first channel to the timer zone, where dye in the label paper reacted with PEG solution, which resulted in the color change of paper from white to red in 2 min, indicating the endpoint of the extraction process. The extraction module included sample area, paper-based channel, Fusion 5 disk and absorbent pad. The filter of sample area and Fusion 5 disk were placed on the second channel which was connected with an absorption pad. Paper-based valve on the third channel, which would be activated by the washing buffer, allowing it to flow into the second channel in manner. When the lysis buffer and washing buffer flowed through paper-based channel, respectively, sample was first lysed by the lysis buffer, then the washing buffer activated paper-based valve to flow from second channel to an absorbent pad through sample area and Fusion 5 disk. The Fusion 5 disk was used to capture the DNA, which is composed of silica-based glass fiber. The working principle of DNA absorption is based on the combination of high affinity between the negatively charged DNA and the positively charged glass fiber [24]. While the absorbent pad was used to absorb the wastes produced by the extraction process, such as the cell debris, hemoglobin and other proteins.

DNA extraction from paper-based DNA extraction device

In our device, *5%* of Tritonx-100 was used to lyse blood, followed by a single wash of 50 mM NaOH to clear the hemoglobin until the disk appeared white [25]. Fusion 5 disk was used to trap the DNA from the lysed whole blood for low-cost and rapid DNA extraction [16, 17, 21]. The device consisted of paper channels with different length that autonomously direct the reagent and sample to flow sequentially to the Fusion 5 disk through capillary action, which represented the unique properties of paper. The entire extraction process was shown in Fig. 2 (see **Supplementary Movie S1** for more details). Briefly, the fingertip was pricked using a lancing device (Fig. 2a–I), 30 μL of blood was then added to a small piece of filter paper connected to the sample area of the device by burette (Fig. 2a–II). After adding the sample, the start button was pressed to initiate the extraction process (Fig. 2a–III), allowing the PEG solution, lysis buffer and washing buffer to simultaneously flow through the first, second and third channels, respectively. Considering different performers may press the start button with different velocity, the accuracy of the timer zone was also assessed. More specifically, the color time of the timer zone was measured under different pressing velocities performed by nine persons. The result shows that the button was pressed down within 5 s for all performers and there is no significant difference in the color time of the timer zone (**Fig. S2**). During the extraction process (starting from t = 0–5 s), the lysis buffer lysed the sample in the sample area. At the same time, the washing buffer activated the paper-based valve on the third channel to connect to the second channel and flowed through both sample area and Fusion 5 disk. The DNA was then captured by the Fusion 5 disk. The wastes including cell debris, hemoglobin and other proteins passed through the disk and were absorbed by the absorbent pad. At t = 120 s, all the PEG solution reached the timer zone (as an endpoint indicator), and the color of the timer zone completely changed from white to red, indicating the endpoint of the extraction process. The Fusion 5 disk containing the DNA was then collected by removing the small piece of paper attached to it (Fig. 2a–IV). The Fusion 5 disk was directly added into a tube for downstream analysis (PCR/qPCR assay).

Optimization of paper-based DNA extraction device

To achieve the high efficiency of DNA extraction, using the DNA of human β-actin as target analyte, the following parameters were optimized, including (a) Distance between area and DNA capture area, (b) DNA extraction periods (e.g., 0, 2, 4, 6 and 8 min), (c) sample volumes (e.g., 10, 30, 50, 70, 90 μL), (d) the volume of reagent used for extraction (lysis buffer-50, 100, 200, 500 μL, washing buffer-100, 200, 400, 1000 μL), (e) the wetting length of different concentrations of PEG8000 solution within 2 min, and (f) the performance of the vacuum-packed extraction device after storing the reagent on chip for 0, 2, 3, 4 and 8 weeks. Respective data and Figures are given in the Supplementary Information. The following experimental conditions were found to give best results: (a) a distance of 0 mm, (b) the DNA extraction periods of 2 min, (c) the sample volume of 30 μL, (d) the reagent volume of 200 μL lysis buffer and 400 μL washing buffer, (e) the wetting length of 6% PEG8000 solution, and (f) the performance of the device can be maintained after the long-term storage (at least 2 months). To assess the effect of longer storage of the reagent, our ongoing work would need the help from a commercial partner in the future, highlighting its potential in real-world applications. (Please see the detail Results and Discussion in the Supplementary Information).

Extraction from biological samples

Paper-based device was utilized to extract different amount of DNA from human whole blood, human serum, human MCF7 cells, human saliva, human sputum, and *E. coli* bacterial suspension, and the detection limit of paper-based device was compared with that of QIAamp® DNA Micro kits and FTA card (Fig. 3). In human blood, the detection limit of paper-based device is 10^{-1} ng, which is higher than that of FTA card (10^{-2} ng) and QIAamp® DNA Micro kits (10^{-3} ng). In human serum, the detection limit of paper-based device is 10^{3} ng, which is similar with that of FTA card (10^{3} ng) and higher than that of QIAamp® DNA Micro kits (10^{-3} ng). In human MCF7 cells, the detection limit of paper-based device is 10^{-3} ng, which is similar with that of QIAamp® DNA Micro kits (10^{-3} ng) and lower than that of FTA card (10^{-2} ng). In human saliva, the

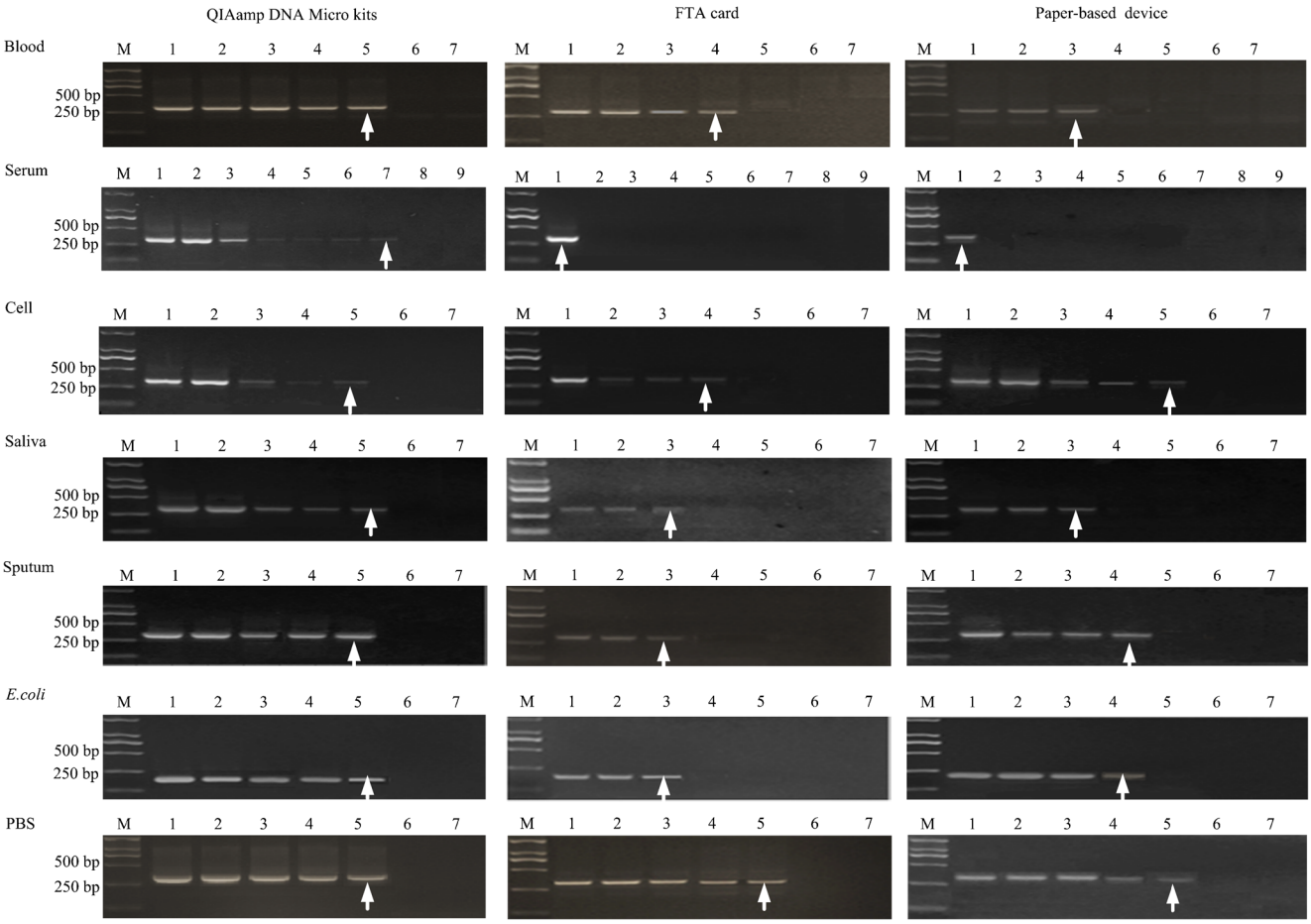

Fig. 3 Comparison of the limit of detection of different samples using three extraction methods. As for serum, 1–10^{3}, 2–10^{2}, 3–10, 4–1, 5–10^{-1}, 6–10^{-2},7–10^{-3}, 8–10^{-4}, 9–0 (ng). For other samples, 1–10, 2–1, 3–10^{-1}, 4–10^{-2}, 5–10^{-3}, 6–10^{-4},7–0 (ng)

detection limit of paper-based device is 10^{-1} ng, which is similar with that of FTA card (10^{-1} ng) and higher than that of QIAamp® DNA Micro kits (10^{-3} ng). In human sputum and *E. coli* suspension, the detection limit of paper-based device is 10^{-3} ng, which is similar with that of the QIAamp® DNA Micro kits (10^{-3} ng) and is lower than that of FTA card (10^{-1} ng). Following the band analysis, the result shows that the higher the intensity of band, the higher the optical density (**Fig. S5**).

Collectively, our data indicate that paper-based device shows different detection limits in detecting target DNA in blood, serum, cell, sputum and bacteria sample. The sensitivity of paper-based device is lower than conventional QIAamp® DNA Micro kits in testing most samples. The detection limit of serum is the highest, which shows that the PCR inhibitor in serum is more than that in other samples. This is because three DNA extraction methods have different working principles, which result

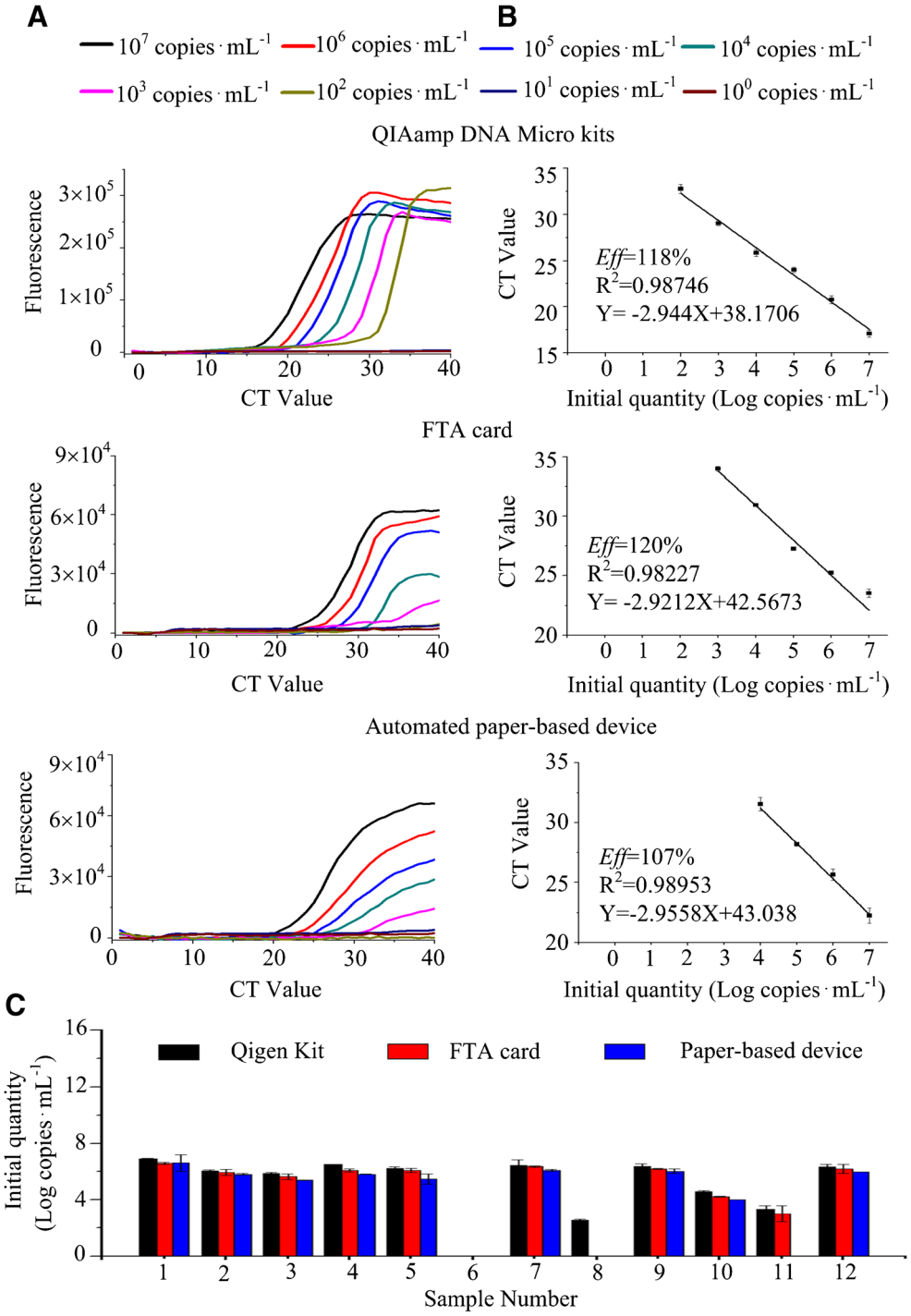

Fig. 4 Clinical samples testing with the paper-based DNA extraction device. **a** Amplification curve of standard positive sample from 10^7 to 10^0 copies·mL^{-1}; **b** Standard curve of average CT vs initial quantity (Log copies·mL^{-1}) obtained with positive control plasmid with 10^7 to 10^0 copies·mL^{-1}, **c** Initial quantity (Log copies·mL^{-1}) obtained with HBV clinical sample after DNA extraction using the QIAamp® DNA Micro kits, FTA card and paper-based device. (*Eff*- PCR efficiency)

in different PCR residues (e.g., undesired protein) that have different inhibition effect on PCR and thus assay [26]. QIAamp® DNA Micro kits used proteinase K to lyse the sample, and salt and alcohol to remove the inhibitors; FTA card used chemical reagent (proprietary) to lyse the sample, and washing buffer and TE buffer to remove the inhibitor [8]; whereas paper-based device used detergent (TritonX-100) and alkali solution (NaOH) to lyse the sample and remove the inhibitors [27]. Hence, the effect of inhibitors was also studied (Please see the Supplementary Information). To improve the sensitivity of this method, our future work would utilize various lysis reagents to enhance the lyse efficiency and use chemical reagent to modify Fusion 5 disk to minimize its absorption of debris protein.

The recovery rate of paper-based DNA extraction device

To investigate the accuracy and reliability of paper-based device, the DNA recovery rate of three DNA extraction methods was measured. The data show that the recovery rates of three extraction methods are different for different biological samples (**Table S2**). For human blood sample and human serum, the recovery rates of FTA card are higher than that of paper-based device. For human MCF7 cells, human saliva, human sputum and E. coli, the recovery rates of FTA card are lower than that of paper-based device. These results demonstrate that the inhibitor of whole blood and serum is more than that of other samples. Additionally, the recovery rate of human MCF7 cell using paper-based device is highest among these different samples. On the contrary, the recovery rate of human serum using paper-based device is the lowest among these different samples. In short, these results show that for most samples, the recovery rate of QIAamp® DNA Micro kits is higher than that of FTA card and paper-based device ($P < 0.05$). The data are in agreement with the result of detection sensitivity (Fig. 3), suggesting that paper-based device with lower recovery rate shows higher detection limit than QIAamp® DNA Micro kits. These might be due to the higher efficiency of conventional processes which requires a longer cell lysis period and multiple washing steps to get a high purity of DNA.

Evaluation of paper-based DNA extraction device with HBV clinical samples

To assess the potential of our device for rapid onsite DNA extraction for nucleic acid-based POCT, in this study, the detection limit of HBV in different buffers using three extraction methods by qPCR was first compared (**Fig. S6**). Additionally, three DNA extraction methods were used to extract DNA (from 10^7 to 10^0 copies·mL^{-1}), and created the amplification curves (Fig. 4a) and the standard curve (Fig. 4b). The data (Fig. 4c) indicate that the limit of detection of QIAamp® DNA Micro kits, FTA card and paper-based device are as low as 10^2 copies·mL^{-1}, 10^3 copies·mL^{-1} and 10^4

Table 1 The advantages and disadvantages of the paper-based extraction device as compared to other methods

Method	Advantages	Disadvantages	Sample volume	Reference
Paper-based device with on-chip reagent storage for DNA extraction	Portable, rapid (within 2 min), on-chip reagent storage in the device, simple operation step	Need to improve the extraction efficiency	30 μL	This study
FTA card	Portable	Not allow reagent storage and require manual addition of reagent onto the paper disk, time consuming (about 1 h)	125 μL	[2, 15]
Filtration isolation of nucleic acid (FINA)	Portable, rapid (within 2 min)	Not allow reagent storage and require manual addition of reagent onto the paper disk	100 μL	[16, 21]
Paper-based origami device	Portable	Need ultrapure water to dissolve the stored dry reagents, which involves multiple washing steps and is time-consuming (about 2 h)	300 μL	[18]
On-chip genomic DNA extraction using magnetics particles	Compatible with micro-system	Need on-chip heater and sensor to control the cell lysing temperatures	10 μL/15 μL	[28, 29]
DNA extraction by laser irradiation and magnetic beads	Portable	Need a higher power laser diode	9 μL	[30]

copies·mL^{-1}, respectively. Then, ten true positives and two true negatives were used to detect the sensitivity and specificity of three extraction methods. The results show that the sensitivity of QIAamp® DNA Micro kits, FTA card and paper-based device are 100%, 100% and 90%, indicating that the sensitivity of our paper-based device is lower than that of QIAamp® DNA Micro kits and FTA card. However, the specificity of QIAamp® DNA Micro kits and FTA card are 50%, 100% and 90%, demonstrating that the specificity of our paper-based device is higher than that of QIAamp® DNA Micro kits and similar with that of FTA card. As mentioned previously, this might be due to the different working principles of each DNA extraction method. In short, we successfully demonstrated that our paper-based DNA extraction device can effectively extract DNA from various biological samples and clinical samples. Comparison of our method using the existing paper-based DNA extraction devices were summarized in Table 1. As compared to FTA card [2, 15], Fusion 5 using FINA [16, 21], paper-based origami device [18], on-chip genomic DNA extraction using magnetics particles [28, 29], DNA extraction by laser irradiation and magnetic beads [30], which do not allow the liquid buffer storage and need multi-steps operation and extra device. Our device allows the liquid buffer storage and achieves DNA extraction (2 min). Besides, it is small and portable (67 mm × 42 mm × 15 mm), requires only a small volume of sample (30 μL), enables on-chip long-term reagent storage (at least two months). These advantageous features make our device well-suited for POC applications.

Conclusion

In this study, a paper-based DNA extraction device was developed by integrating sponge-based reservoir module, paper-based valve, paper-based extraction module and paper-based timer into one platform. This device can extract DNA from various biological samples, including human whole blood, human serum, human MCF7 cells, human saliva, human sputum and *E. coli* suspension within 2 min without external equipment and complex operations, which can be directly used for downstream analysis, such as PCR and qPCR. In short, the paper-based DNA extraction device offers many advantages, including 1) low cost due to the use of common type of paper and chemicals (**Table S3**); 2) rapid extraction, in which short extraction time is required (2 min); 3) capability of extracting DNA from various biological samples. However, the limitation of paper-based device is the lower extraction efficiency, our future work would improve it by utilizing various lysis reagents to enhance the lyse efficiency and chemical reagent to modify Fusion 5 disk to minimize its absorption of debris protein. In the future, we envision that this device can be fully integrated into a paper-based amplification and detection platform (e.g., PCR and lateral flow assay), offering great potential for disease diagnosis, food safety analyses and environmental monitoring.

To ensure the accuracy and the cross-contamination of this assay without being affected by environmental factors (e.g., extremely hot or cold, and dry or wet environments, or contaminated environment), future work should focus on development of a portable closed system with precise temperature-humidity control to provide a contamination-free optimum condition for DNA extraction, amplification and detection [31]. If the extracted DNA is not immediately used for the next analysis, it can be stored in the contamination-free Eppendorf tube in order to avoid cross-contamination.

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Compliance with ethical standards The author(s) declare that they have no competing of interests.

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