NON-CENTRIFUGAL MICROFLUIDIC NUCLEIC ACID TESTING ON LAB-ON-A-DISC

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ABSTRACT

Most lab-on-a-disc platforms use centrifugal force to pump liquids. In this work, we demonstrated a novel energy efficient non-centrifugal lab-on-a-disc nucleic acid testing device in which no liquid motion is involved. Streamlined DNA extraction, amplification, and real-time detection on a single microfluidic reagent disc are achieved by actuating the DNA-carrying magnetic beads against stationary reagent droplets. Using malaria spiked whole blood as a testing model, we demonstrated an excellent detection limit of 0.5 parasites/µl, sufficient for detecting asymptomatic malaria parasite carriers.

KEYWORDS

Nucleic acid tests, malaria, lab-on-a-disc, point-of-care, streamlined sample preparation, non-centrifugal.

INTRODUCTION

Malaria is a mosquito-borne infectious disease caused by Plasmodium parasites. Among five malaria species, *P. falciparum* and *P. vivax* are the most virulent ones, responsible for 219 million cases world-wide annually [1]. Although growing malaria control efforts reduced the malaria incidence rate over the past decade, malaria elimination and eradication remain a challenging goal [1]. The reason is that effective malaria elimination requires the identification and treatment of both symptomatic and asymptomatic carriers to eliminate the source of malaria transmission [1]. Therefore, effective malaria elimination strategies rely on highly sensitive, specific, and mass malaria screening [2]. Conventional malaria screening tests rely on microscopy and immunological rapid diagnostic tests (RDTs) with a detection limit of 50 – 100 parasites/µl [3]. Both methods work well with the high transmission region where the symptomatic patients are dominant. However, this level of sensitivity is insufficient for identifying asymptomatic carriers in low transmission area since their parasite load is below 5 parasites/µl [4].

As an alternative, nucleic acid tests (NATs) have been used for detecting low-level asymptomatic infections since its detection limit is approximately 1 parasite/µl [5]. Among various NATs (*e.g.*, polymerase chain reaction (PCR), recombinase polymerase amplification (RPA) [6], and helicase dependent amplification (HAD) [7]), loop-mediated isothermal amplification (LAMP) has emerged as a promising technology for field use due to its simplicity, rapidness, sensitivity, and specificity. Besides, it has been well established for malaria identification. Despite its potential, most LAMP-based detection is still limited since it requires expensive and bulky peripheral equipment as well as a skilled technician for manual sample process. Moreover, basic infrastructures (*e.g.*, electricity) are often lacking in a resource-limited setting [8]. To bring the NATs to the field, the test assay has been incorporated with various microfluidic-based lab-on-chip technologies [9]. However, one of the grand challenges for nucleic acid testing (NATs) at the point of care is related to the front end of the assays - nucleic acid extraction from raw samples. The ideal sample preparation should be simple, scalable and easy-to-operate. Prior work on lab-on-a-disc platforms for NATs exclusively utilized centrifugal forces to move liquid to the desired location in the microfluidic disc [10, 11]. While it works great for various applications, the centrifugal forces are energy-hungry.

In this work, we demonstrate a non-centrifugal method to move the nucleic acid bearing magnetic beads by energy efficient magnetic interaction to the desired liquid chambers on the microfluidic disc. This innovation enables a unique method for high-quality nucleic acid sample preparation and detection on a single enclosed microfluidic reagent disc. It greatly simplifies the complex DNA analysis and offers low-cost and accurate diagnosis performance in a quick and automated fashion. The device could deliver sensitive (~0.5 parasites/µl) NAT results directly from a small volume of whole blood samples within 50 minutes.

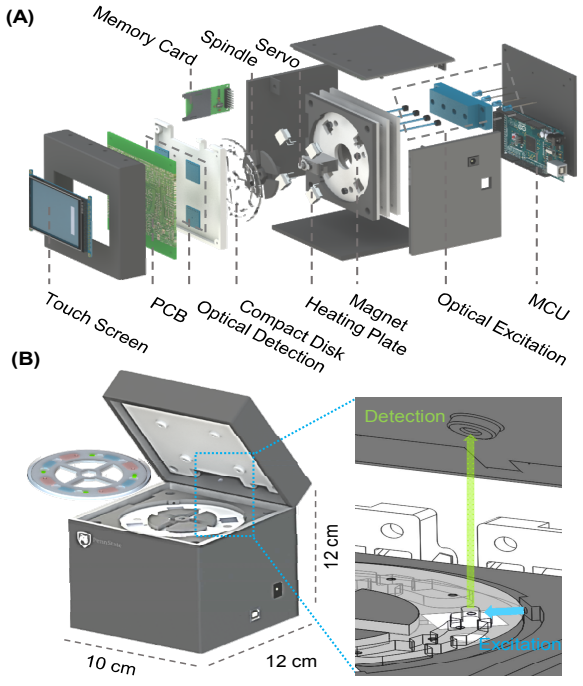

Figure 1: Overview of the analyzer. (A) Exploded view showing the assembly of various components. (B) Schematic of the assembled device and the microfluidic reagent compact disc. The form factor of the analyzer is palm-sized. The reagent compact disc is secured to the spindle platter. A real-time fluorescence sensing scheme is integrated into the analyzer.

RESULTS

Ultracompact and portable analyzer

Fig. 1A shows the real-time fluorescence analyzer. The analyzer has a small footprint of 10×12×12 cm^3. The fully integrated analyzer consists of thermal, optical, electromechanical and data subsystems (Fig. 1B). To maintain the constant temperature (65°C) at each testing unit during the DNA amplification process, four resistive-heating elements were connected in series and controlled by negative thermal feedback. Four blue LED light sources, and optical sensors were used to real-time monitor the DNA amplification. Four blue LED light (λ=488 nm) were aimed at the individual reaction chambers for excitation. The optical sensors were located perpendicular to the incidence of the excitation light to reduce the interference from excitation light (Fig. 1B). The entire system was powered by a 9V portable Lithium-ion battery. Embedded microcontroller unit (MCU) operated a streamlined sample process, real-time data analysis, and remote/on-site result report. The total cost of the portable analyzer was approximately $200. It could be easily built or repaired in any ordinary laboratory setting.

Workflow

The testing procedures consist of the following four steps: 1) The 20 µl of finger-prick blood was collected in a capillary tube and lysed in the collection tube, which contains 1000 µl of lysis buffer. 2) 180 µl of lysate was introduced the binding chamber in the reagent disc (Fig. 2A) and sealed it with PSA tape to prevent evaporation during the thermal process. 3) Insert the prepared reagent disc to the analyzer for the streamlined nucleic acid sample process. During this process, nucleic acid sample preparation (10 min) and amplification (40 min) were performed without user intervention. 4) The real-time relative fluorescence unit (RFU) data were processed by a built-in algorithm and plotted on the LCD screen. The data were also stored in the SD memory card for the record. Users also have the option to receive the results through a smartphone.

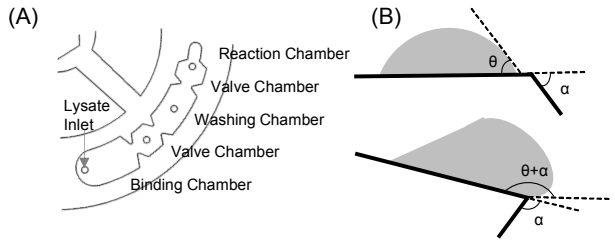

Figure 2: (A) A unit test on the microfluidic disc. The testing unit consists of binding, washing and reaction chambers, isolated by the valve chambers. (B) Illustration for the structural pinning effect.

Robust passive liquid valve (PLV).

The proposed system incorporates the microfluidic reagent disc, which actuates the DNA-binding magnetic beads into the stationary reagent droplets in each functional chambers. We introduced the teeth-shaped passive valves between the reagent chambers to securely hold and to separate the liquid (Fig. 2A). The passive valve uses structural pinning effect and modified surface tension (Fig. 2B). The pinning effect refers to the fact that a sharp bending angle (α) of the teeth structure radically increases the liquid/vapor interface area and raises the activation energy, which increases the barrier of the fluid [12, 13]. The valve surface was also modified by water-oil repellent to increase activation barrier. The robustness of the passive valve for preventing the reagents from mixing and cross-contamination under the harsh mechanical vibration was validated by drop test and hand agitation.

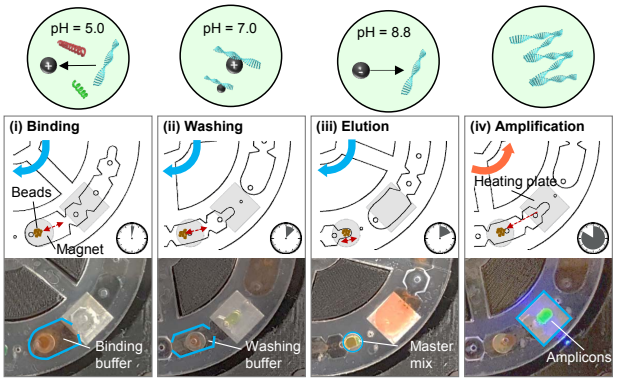

Figure 3: Sample preparation and amplification steps on the disc. By rotating the disc against a stationary magnet, the pH charge switchable magnetic beads were directed from chamber to chamber.

Charge switchable magnetic bead-based DNA isolation.

Non-centrifugal streamlined sample preparation was achieved by magnetic interaction method using the pH-sensitive charge-switchable magnetic beads (ChargeSwitch Forensic DNA Purification Kits, Invitrogen). Briefly, the process for each sample consists of the following three steps: binding, washing, and elution (Fig. 3). The negatively charged parasite DNAs bind to the positively charged magnetic beads at pH 5 (3 min). The reagent compact disc was rotated clockwise and counterclockwise to capture parasite DNAs in the lysate. The DNA-binding magnetic beads were transferred to the washing chamber by magnetic actuation. During the washing steps (4 min), DNAs were purified to remove the interfering substances. The purified DNAs were further transferred to the reaction chamber where the LAMP master mix is loaded. Since the LAMP master mix has a pH of 8.8, the surface charge of the magnetic beads was switched to the negative. The negatively charged DNAs were then released from the magnetic beads and eluted into the master mix. Finally, the residual magnetic beads were removed from the reaction chamber by disc rotation before the LAMP reaction. Within 10 minutes, the entire sample preparation for four samples was completed in parallel with minimal user intervention. The throughput of the multiplexed sample processing is easily scalable for mass malaria screening.

Quantitative and uniform fluorescence sensing

Fluorescence sensing uniformity is essential for the quadplex parallel measurement. To test the fluorescence sensing consistency at four channels, we introduced

identical calcein aliquots in the reaction chamber. The measurement was repeated with various calcein concentration. The narrow RFU distribution confirms quantitative uniformity among four channels with small standard deviation (Fig.4A). To further validate the sensing uniformity, we performed LAMP reactions in parallel with *P. falciparum* genomic DNAs sample at a single concentration. A small variation of the threshold time (T_t) among different reaction channel validates the sensing uniformity (Fig. 4B).

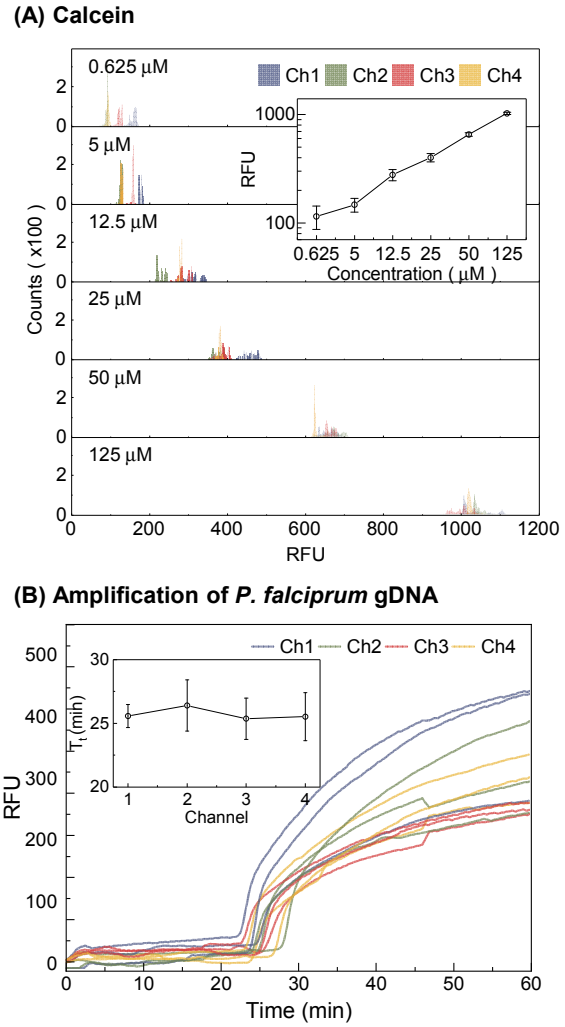

Figure 4: Validation of the optical sensing uniformity. (A) Calcein dye. (B) P. falciparum genomic DNA.

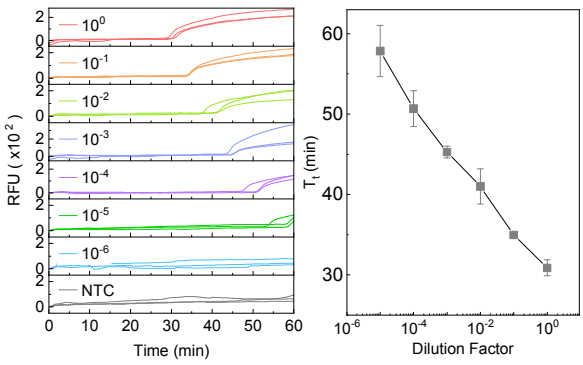

Figure 5: Validation of quantitative capability of the optical sensing. 10-fold serially diluted P. falciparum genomic DNA samples was used.

Finally, we performed LAMP reaction using serial diluted *P. falciparum* genomic DNAs to demonstrate the quantitative capability of the fluorescence sensing. We observed the linear relationship between T_t and dilution factor, which can be used as a standard curve to quantify the sample concentration (Fig. 5). This quantitative ability enables potential usage of parasite load assessment [14].

Sensitivity test

The analytical sensitivity was evaluated using lab-cultured *P. falciparum*-infected whole blood sample with various parasitemia. The four testing units on reagent compact disc were configured as three aliquots of the testing samples and one negative control. The loaded samples (lysate containing either parasite DNA or no template) were automatically prepared on the reagent compact disc in parallel. As shown in Fig. 6, a whole blood sample with parasitemia higher than 10^{-5}% (*i.e.*, 0.5 parasites/µl) could be identified using microfluidic reagent compact disc and analyzer. This level of detection limit satisfies the WHO requirement (*i.e.*, 2 parasites/µl) for identifying early-stage asymptomatic carriers in a pre-elimination setting [1]. The integrated, streamlined sample preparation is capable of extracting high-quality *P. falciparum* DNAs without the interference of human genomic DNA from an infected whole blood sample. The threshold time for each amplification result was plotted against the parasitemia (Fig. 6B). Similar to the experimental results using *P. falciparum* genomic DNA samples (Fig. 5), inversely proportional relationship is valid for whole blood samples with integrated sample preparation (Fig. 6B). However, the curves are not exactly linear. It is noteworthy that the magnetic bead-based DNA extraction is nonlinear for low parasitemia samples, which is likely due to the DNA loss during the sample processing [15].

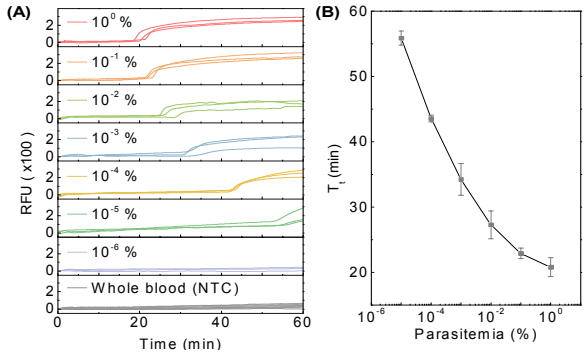

Figure 6: Sensitivity test with P. falciparum-infected whole blood sample Amplification curves for 10-fold serially diluted P. falciparum-infected whole blood samples.

Specificity test

To validate the species-specific detection capability of the system, we prepared the *P. falciparum* and *P. vivax* spiked whole blood. For the specificity evaluation, four testing units on the reagent compact disc were configured as species- and genus-specific tests (*i.e.*, *P. falciparum*-specific, *P. vivax*-specific, *genus*-specific, and *genus*-specific for no-template control). The spiked whole blood

was lysed and loaded into each testing unit for the automated quadplex sample processing. 100 RFU was experimentally set as a threshold for distinguishing positive and negative results. As shown in Fig. 7, we were able to identify two of the most life-threatening malaria species (*P. falciparum* and *P. vivax*). The classification of a particular infection was derived from qualitative results on the single reagent disc. For example, *P. falciparum*-infection can be classified from the positive results in *P. falciparum*-specific test and genus-specific test (The first row of Fig. 7). Mixed infection sample can be detected when *P. falciparum*-, *P. vivax*-, and *genus*-specific tests show all positive. Identification of the most vital human malaria species (*P. falciparum* and *P. vivax)* could provide malaria transmission profiles and enable effective elimination strategy in the field [9].

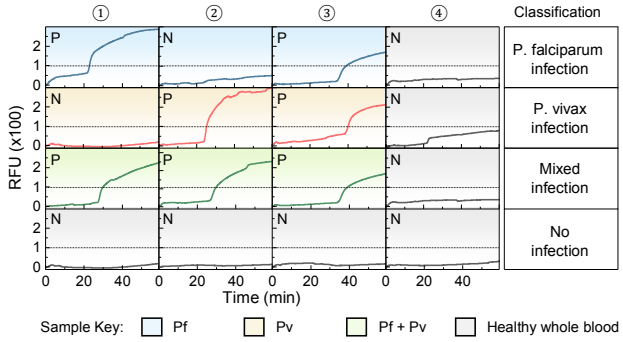

Figure 7: Species- and genus-specific tests using spiked whole blood samples.

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