# FULLY INTEGRATED MOLECULAR DIAGNOSTCS OF PATHOGENIC MICROORGANISMS ON A DISC

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## ABSTRACT

A fully integrated molecular diagnostic assay for pathogen detection is demonstrated on a centrifugal microfluidic device. Staring from a large volume of sample ( ~ 1 mL), a series of reaction from target enrichment, DNA extraction, polymerase chain reaction (PCR), and fluorescence detection were fully automated on a disc. To achieve a stable thermocycling on a disc, a novel laser-mediated non-contact heating and thermally stable reversible valving techniques were developed. As a proof of concept test, a "sample-in and answer-out" type of the pathogen detection was demonstrated using a gram-positive bacteria sample, *Staphylococcus aureus*.

**KEYWORDS:** Molecular diagnostics, Lab-on-a-disc, PCR

## INTRODUCTION

Molecular diagnostics is a sensitive and fast technique for detecting pathogenic microorganisms and determining optimal treatment for related diseases. Polymerase Chain Reaction (PCR) is an essential technique in molecular diagnostics, and it facilitates highly sensitive disease detection with good specificity. However, there is no point-of-care-test (POCT) type PCR-based pathogen detection system available yet because it requires a laborious process with expensive equipment. Previously, we have demonstrated an integrated molecular diagnostic device for food-borne pathogen detection using isothermal amplification and laser based non-contact heating[1]. Developed heating technique reduces power consumption and complexity of the system configuration. Here, we report a lab-on-a-disc for a fully integrated PCR and it is attributed to the combination of three novel techniques; the wireless local heating by laser diode, precise metering of the lysate using oil replacement method, and the thermally stable and reversible valving technique.

## EXPERIMENTAL

Figure 1(a) illustrates the microfluidic channels on the disc designed for a fully integrated pathogenic bacteria detection. A disc consists of 9 chambers for sample, washing buffer, oil, lysis buffer, PCR

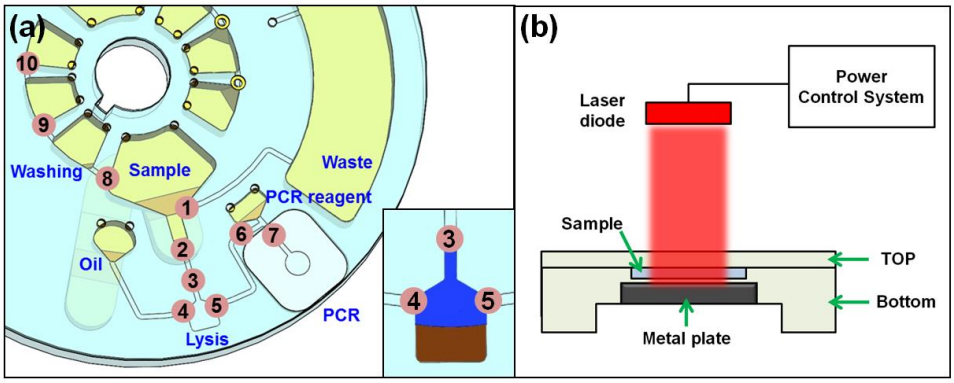


*Figure 1: (a) The layout of the disc for molecular diagnostics of pathogenic bacteria. Inset is the detailed design of the lysis chamber; the blue area was transferred to the PCR chamber while that in brawn area was remained in lysis chamber.(b)Detailed configuration of the laser-mediated non-contact heating method.*

reagent, amplification reaction, and waste storage and 10 reversible valves. The details of the fabrication method can be found elsewhere[1, 2]. In the novel valving technique for reversible flow control, a pair of bolt and nut was assembled to squeeze or release an epoxy layer. No materials were consumed during the actuation and the reversible actuation was possible. Figure 1(b) represents the configuration of the non-contact heating for the thermocycling. Aluminum plate was attached on the backside of the PCR chamber, and it worked as a heater when the laser was activated. A 808 nm laser diode was utilized and the power of the laser was continuously controlled by a computer system. We calibrated a temperature profile first and used the corresponding power program for the heating process for the PCR. The pre-annealing was conducted for 5 min at 95°C and 40 cycles of denaturation/annealing at 95°C for 10 s and the extension at 64°C for 30 s were repeated.

**RESULTS AND DISCUSSION**

For the disc operation, 1 mL of the sample solution containing the magnetic bead coated with S. aureus specific antibody and 2 μL of PCR reagent are introduced to the sample chamber and the PCR reagent chamber, respectively (Figure 2(a)). The incubation for 10 min is performed with shaking a disc in order to capture target pathogens with magnetic beads. After the incubation, an embedded magnet is moved to the inner position using the customized magnet control system and subsequent rotation of the disc leads to collection of beads on the bottom of sample chamber. (Figure 2(b)). Then, supernatant of the sample solution is discarded after opening the valve #4 as illustrated in Figure 2(c) and the first washing solution is transferred to the sample chamber by opening the valve #3 as shown in Figure 2(d). Sediment magnetic beads on the bottom part can be moved to the sample chamber again by manipulation of the magnet inward (Figure 2(e)). Otherwise, packed magnetic beads by strong centrifugation at previous step are unable to be dispersed on sample chamber and it results in poor washing efficiency. After washing the beads with shaking mode for 30 s, the supernatant is removed again by opening the valve #4 (Figure 2(f)). The washing was performed twice to remove sample residuals, then the magnetic beads are moved to the lysis chamber as shown in Figure 2(g). Lysis is carried out by activation of the laser diode for 20 s and

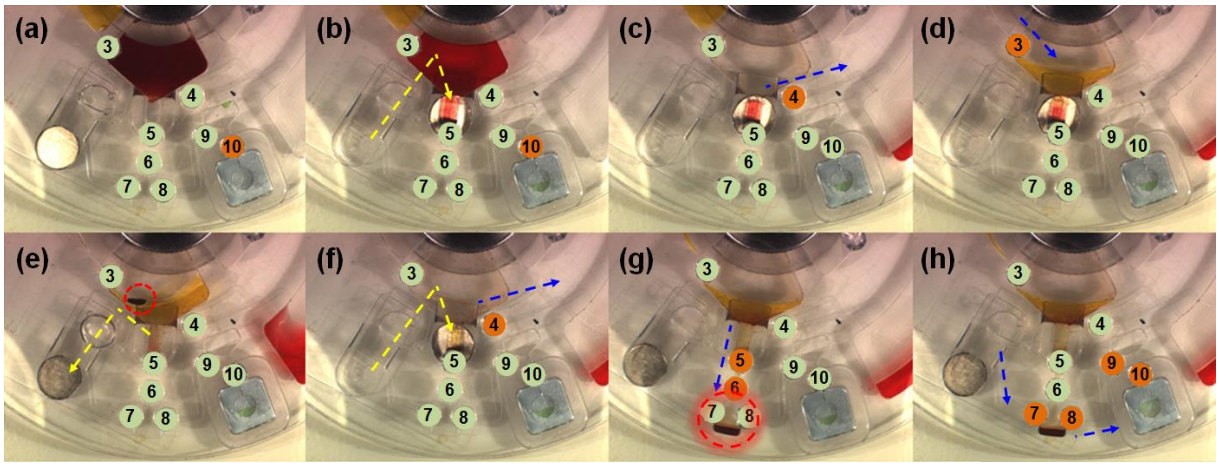


*Figure 2: Images showing the sequential operations of the disc for a fully integrated bacteria detection (Dyed solution was utilized here for better visualization): (a) A sample introduction and incubation, (b) the collection of magnetic beads by rotation of a disc and a magnet, (c) the removal of the supernatant, (d) the introduction of washing buffer, (e) the movement of the beads to the sample chamber (Red dot circle) for washing by a magnet. (f) the removal of the supernatant. (g) beads transfer and bacteria lysis by the laser irradiation. (h) lysate metering by the oil replacement and PCR. In all illustrations, green and orange dots represent the normally closed valves and normally opened valve, respectively.*

finally, valves #7, #8, #9 and #10 are open and the disc is rotated to perform the metering by oil replacement. As the disc rotates, oil is transferred from the oil chamber to the PCR chamber along the microfluidic channel, replacing the lysate solution that is located in the upper part of the lysis chamber (blue area in the inset of Figure 1(a)), while magnetic beads were remained in the bottom of the lysis chamber as presented Figure 2(h). Graphical analysis demonstrated that 3.2 μL of supernatant lysate was moved to the PCR chamber with low variation in volume (5.6 % of CV). The transferred lysate was mixed with pre-loaded PCR mixture, and the laser heating was conducted after the closure of the valve #4. Figure 3 shows the result of a fully integrated PCR on a disc using $10^4$ cfu/mL of *S. aureus* spiked in PBS. Significantly increased fluorescence signal and the clear band signal in the positive sample (P) demonstrated the successful amplification on the integrated disc.

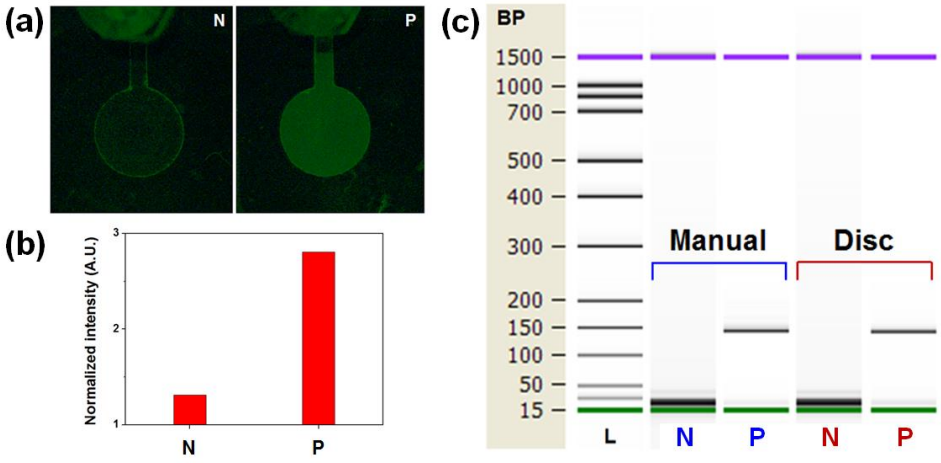


*Figure 3: PCR on a disc is confirmed by (a) the fluorescence image, (b) the fluorescence signal intensity, and (c) gel-electrophoresis. Data with blue color is from the manual steps and red color is from the disc. N and P notes for negative control and the positive sample, respectively.*

## CONCLUSION

Overall, we demonstrated a sample to answer type device for molecular diagnostics of the pathogenic bacteria and the entire process could be, for the first time, fully integrated and automated on a small device. Our compact and miniaturized platform could be a powerful tool for prompt molecular diagnostics in the fields where POCT is desired.


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