MINIATURIZED PCR DEVICE FOR RAPID DETECTION OF INFECTIOUS AGENTS

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ABSTRACT

This paper reports a new point-of-care testing (POCT) device concept for rapid detection of infectious agents. Recently, accurate and rapid diagnosis of infectious diseases has become critical to preventing their spread, but genetic-based tests are very complex and considerable time is required to check many samples. Therefore, a genetic based tester, which subjects samples to rapid thermal cycling and enables the sample solution to be easily injected, is desired. This paper reports our micro heater-based POCT system and presents a rapid multiple target detection result.

KEYWORDS: Point-of-care testing , Polymerase chain reaction, Influenza, Polydimethylsiloxane

INTRODUCTION

Recently, a portable diagnostic device for realizing POCT in various fields has been desired. Accurate and rapid diagnosis of infectious diseases is especially critical for preventing disease spread. POCT devices must be compact and have high speed to test samples in the field [1, 2]. However existing portable devices are based on low-sensitivity immunochromatographic strips, whereas desktop systems are sensitive and automatic but heavy and consume much power. Nucleic-acid amplification tests using the polymerase chain reaction (PCR) are widely used to test for infectious diseases. Unfortunately, PCR-based tests are very complex and considerable time is required to check many samples. Therefore, a PCR-based tester, which subjects samples to rapid thermal cycling and which enables the sample solution to be easily injected, is desired. Additionally, it is crucial to select a manufacturing technique and disposable chip that can be fabricated at low cost to avoid any cross contamination between different samples [3].

In this report, we describe our miniaturized PCR system that consists of nine independent micro heaters, an LED and a photodiode array. The module's heating system facilitates fast thermal cycling because of the improved heat-transfer efficiency achieved by applying a small amount of reaction solution and a micro heater. Furthermore, we also report our novel vacuum-aided microfluidics [4]. Finally, the results of infectious agent detection are presented.

EXPERIMENTAL

Figure 1 depicts the POCT system with laptop computer (VGN-P61S, Sony, Japan). Our miniaturized rapid thermal-cycle module consists of nine individual 1.2 mm square heaters, each with its own temperature sensor (Fig. 2). A cross-section of the thermal-cycle module with an LED array and photodiode array for real-time measurements is presented in Fig. 3. Specifications for the LED and photodiode arrays are optimized to detect fluorescence of 6-FAM dye (Ex=494 nm, Em=518 nm). The module has a chip with nine wells connected by channels (Fig. 4). The Polydimethylsiloxane (PDMS) chip contains nine wells, and each well is 1.4 mm in diameter and 0.65 mm deep and holds 1 μL of sample liquid. The PDMS chip dimensions are 30 mm×15 mm×1.6 mm, and it consists of three layers: a PDMS structure layer sandwiched between a glass substrate layer and a glass cover layer.

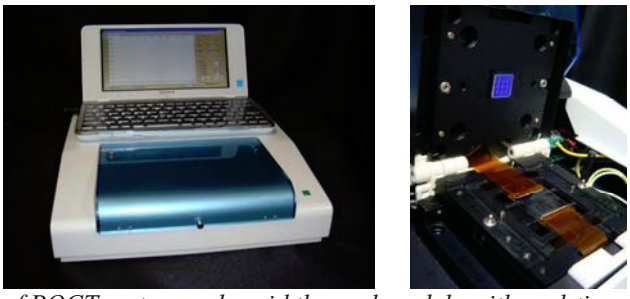

Figure 1: View of POCT system and rapid thermal module with real-time detection system

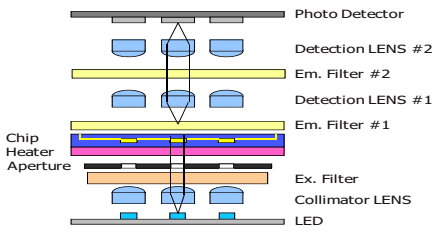

Figure 2: Cross-section of heating and real-time detecting module

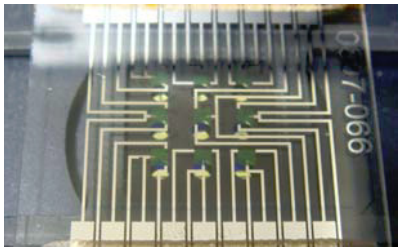

Figure 3: Photograph of heater substrate with arrayed micro-heaters and temperature sensors

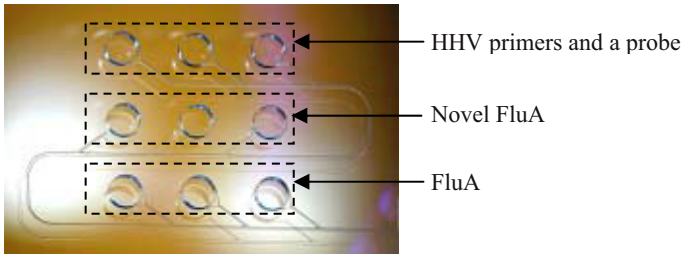

Figure 4: Photograph of reagent preloaded micro-fluidics chip substrate

We selected TaqMan probe assay and prepared three primers and probe sets to detect influenza A (FluA) [5] and novel swine influenza A virus 2009 detected in April 2009 (Novel FluA) [6]. Primers and a probe set for human herpes virus (HHV) [7] detection were also prepared as negative controls (see Table 1). Following this, different primers and probes sets were manually loaded into wells using dispense robots. We preloaded and dried three different primers (0.4 pmol/well) and probe (0.2 pmol/well) sets on the bottom of each well. The reaction mixture consisted of 1×Z-Taq buffer, 0.2 mM each dNTPs, 4.5 mM $MgCl_2$, 2.5 units/uL Z-Taq DNA polymerase (TAKARA Bio, Inc., Japan) and plasmid DNA standard for Flu A (1×10^7 copies/μL).

The sample solution was easily injected into the nine wells using a vacuum-aided injection method and flowed through the channel (Fig. 5).

Table 1. Sequence details of all primer-probe combinations used

Virus type	Target gene	Oligonucleotide sequence (5′–3′)	Product size
Novel FluA	N1	GTTAACATCAGCAACACCAACTTTG	73 bp
		GAGAGGAATTGCCCGCTAATT	
		6-FAM-TGCTGGACAGTCAGTGGTTTCCGTG-**Iowablack**	
FluA	Matrix protein	GACCRATCYTGTCACCTCTGAC	105 bp
		AGGGCATTYTGGACAAAKCGTCTA	
		6-FAM-TGCAGTCCTCGCTCACTGGGCACG-**Iowablack**	
HHV	DNA polymerase	GAAGCAGCAATCGCAACACA	74 bp
		AACCCGTGCGCCGCTCCC	
		6-FAM-ACAACATGTAACTCGGTGTACGGT-**Iowablack**	

We examined the PCR amplification with the following thermal cycling profile: initial denaturation at 95 deg C for 5 s; followed by 40 cycles of denaturation at 95 deg C for 2 s, and annealing/extension at 60 deg C for 9s, corresponding to 10 min 5 s required to complete 40 cycles of PCR. The nine wells were filled with plasmid DNA sample solution using vacuum-aided injection method. TaqMan probe assay was used for monitoring the increase of PCR product.

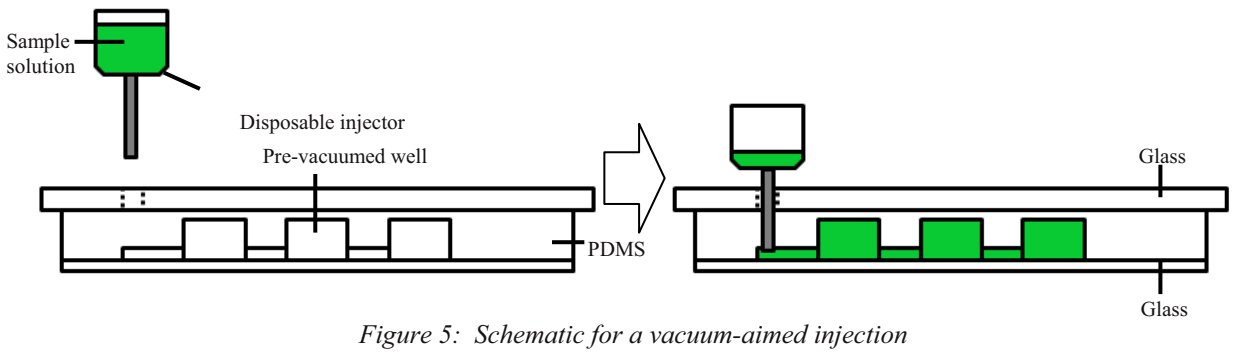

Figure 5: Schematic for a vacuum-aimed injection

RESULTS AND DISCUSSION

We succeeded in easily injecting samples by the vacuum method and rapid PCR with a micro heater array and PDMS chip. Figure 6 plots the result of a typical real-time PCR amplification curve. Data of three wells, which were preloaded with FluA primer and a probe set, compose the PCR signal curve. The signals of the other six wells, which were preloaded with Novel FluA and HHV primers and probe, are flat. These results indicate that our novel POCT system can detect infectious agent rapidly and simultaneously identify gene subtype. We have another chip that has 200 nL wells and it takes less than 6 min to complete 40 thermal cycles (data not shown).

In this report, we used only plasmid DNA because this study's aim was to prove our POCT system concept. Of course, we can perform reverse transcript PCR with our POCT system. If we combine our POCT system and easy extraction method or reagent (e.g. Ampdirect, Shimazu Co., Japan) , it will be possible to realize true POCT.

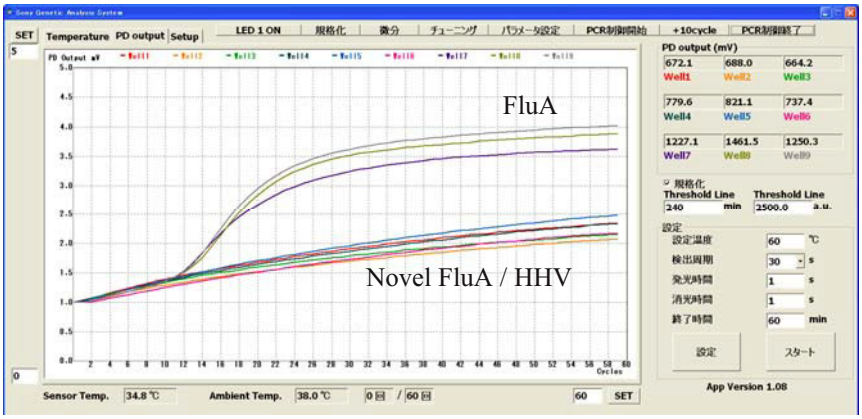

Figure 6: Graph of real-time PCR amplification of swine influenza by POCT device

CONCLUSION

The study results demonstrate that our POCT system facilitates easy sample injection and reduced sample preparation time. The POCT device demonstrated a rapid response for simultaneous multiple-target detection and achieved rapid thermal-cycling, which is a promising step toward developing a fully integrated device for POCT.

Finally, we believe that this device can be applied widely to various fields in life science including clinical, research, environmental and agricultural analysis applications.

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