Eight-chamber microfluidic device with integrated loop mediated isothernmal amplification (LAMP) for multiple detection of Campylobacter spp from pig at slaughter

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EIGHT-CHAMBER MICROFLUIDIC DEVICE WITH INTEGRATED LOOP MEDIATED ISOTHERNMAL AMPLIFICATION (LAMP) FOR MULTIPLE DETECTION OF *Campylobacter spp* FROM PIG AT SLAUGHTER

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ABSTRACT

We describe a Lab-on-a-chip (LOC) device consists of a polymeric injection molded eight-chamber chip and an optical reader in combination with Loop-mediated isothermal amplification (LAMP) for rapid multiple detection of *Campylobacter* spp- a most common food-borne pathogens – directly from pig at slaughter enrichment samples. By integrating LAMP on the LOC which can handle eight samples at one time and reduces the detection time from 5 days by culture or one day by conventional real-time PCR to 30 minutes.

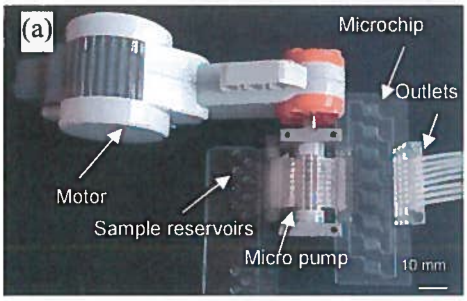
KEY WORDS: Lab-on-a-chip, Isothermal amplification, LAMP, Campylobacter, Foodborne Pathogens

INTRODUCTION

Food safety is one of great concerns of public. According to Centers for Diseases Control and Prevention (CDC), more than 250 pathogens and toxins are known to cause foodborne illness. In United States it was estimated of 48 million foodborne illness. It was estimated 48 million foodborne illnesses occur every year resulted in 55,961 hospitalization and 1,351 deaths. Among these diseases, campylobacteriosis is one of the most common food-borne diseases [1]. In The EU, Nine million human campylobacteriosis cases per year are estimated [2]. Therefore rapid and reliable methods for detecting of Campylobacter are urgent needed. We describe for the first time a Lab-on-a-chip (LOC) device consists of a polymeric injection molded eight-chamber chip and optical reader in combination with Loop-mediated isothermal amplification (LAMP) for rapid multiple detection of *Campylobacter* in pig at slaughter enrichment samples.

EXPERIMENTAL

Figure 1(a) shows the setup of the LOC device. The LOC consists of a disposable polymer chip - 8 reaction chambers connected with inlet and outlet microfluidics (76 mm/length/ × 26 mm/width/ × 1 mm/height/) - fabricating by injection molded on cyclic olefin copolymers (Topas® COC) slide. An external heater element mounted under the microchip and a plastic frame containing 8 magnetic elements was clamped on top of the chips for on chip sample preparation and on chip PCR amplification. Sample reservoirs were connected with the eight chamber micro-device through an eight channels peristaltic micropump. The PCR products are read out by using a simple light transmission optical setup showing in Figure 1(b), which consists of a collimated LED light source and USB microscopy.



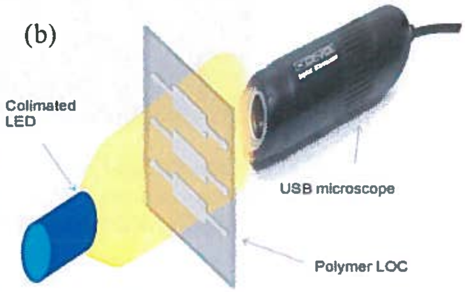


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Figure 1: (a) The LOC system consists of an eight-chambers microfluidic biochip made by injection molded COC slide, an external heater element mounted under the microchip and a plastic frame containing 8 magnetic element was clamped on top of the chips for on chip sample preparation and on chip PCR amplification. Sample reservoirs were connected eight chamber micro-device through an 8 channels peristaltic micropump. (b) Optical read out setup monitoring transmission light from the sample - the most sensitive method for the transparent sample in the solution

RESULTS AND DISCUSSION

On chip sample preparation was performed using 10 μl of enrichment pork meat at slaughter in Buffered Peptone Water (BPW) [3] and 5 μg/ml super-paramagnetic beads suspended in lysis buffer. The sample was loaded into each chamber at a flow rate of 5 μl/min. The DNA - magnetic bead complexes were washed (2 min) and kept inside the chamber by the magnet system. After sample preparation, 10 μl LAMP mixture consisting of different primer pairs targeting the Oxidoreductase gene of *Campylobacter jejuni* and the Aspartate kinase gene of *Campylobacter coli* (Eiken chemical Ltd Japan) was pumped to the PCR chamber. In an initiated experiment, we tried to find the minimum time for an on chip LAMP isothermal amplification. The LAMP was tested for 15, 30, 45 and 60 min. Figure 2 shows the results of one of such experiments. No different in LAMP amplification for 30, 45 and 60 min were observed therefore on chip LAMP isothermal amplifications at 65 °C for 30 min following an inactivation at 82 °C for 2 min were conducted in all the experiments.

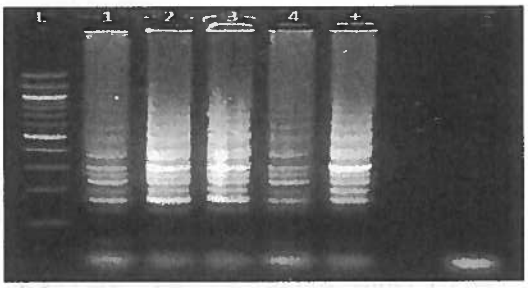

Figure 2: 2% agarose gel electrophoresis end point analysis for on chip sample preparation and LAMP isothermal amplification using the eight-chamber Lab-on- a-chip systems. On gel, L: 100bp DNA ladder, lane 1: 15min and 2ng, lane 2: 30min and 2ng, lane 3: 45min and 2ng, lane 4: 15min and 1ng, (+): positive control in 30min and (-): negative control.

Figure 3 shows the results of on chip sample preparation and LAMP isothermal amplification of pig at slaughter enrichment sample spiked with 2ng, 20pg, 2pg, 1pg and 0,1pg DNA of *C. jejuni* per reaction. Using the LOC device, the limit detection of the test was determined as low as 1pg/reaction.

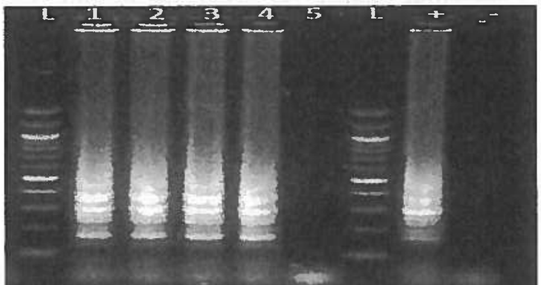

Figure 3: 2% agarose gel electrophoresis end point analysis for on chip sample preparation and LAMP isothermal amplification using the eight-chamber Lab-on- a-chip systems. On gel, L: 100bp DNA ladder, lane 1, 2, 3, 4, 5: 2ng, 20pg, 2pg, 1pg and 0,1pg of Campylobacter jejuni DNA per reaction, respectively, lane + and -: positive and nagative control.

Figure 4 showed the results of one of the experiments using the eight chamber LOC device for rapid detection and identification of *Campylobacter* spp. in pig meat enrichment samples at slaughter. It is very clear as the results for the two pig meat samples that were positive for Campylobacter spp (c and b) in comparison to Negative control (a) and positive control (d).

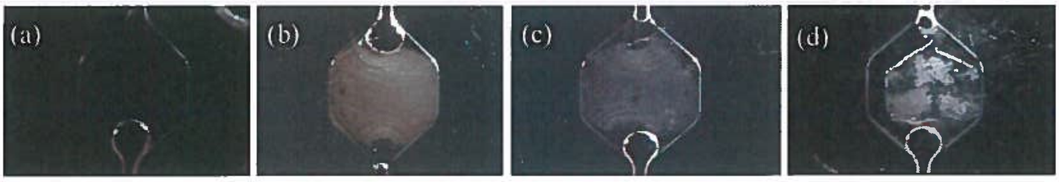

Figure 4: On chip LAMP amplification optical detection. In (a) Negative control; (b) and (c) LAM positive pig meat samples and (d) Positive control.

CONCLUSION

Using the polymeric injection molded eight-chamber chip and an optical reader in combination with Loop-mediated isothermal amplification (LAMP) we are able to detect *Campylobacter* spp - a most common food-borne pathogens – directly from pig meat at slaughter enrichment samples. By integrating LAMP on the LOC which can handle eight samples at one time and reduces the detection time from 5 days by culture or one day by conventional real-time PCR to 30 minutes. This novel LOC device is a good tool for rapid clinical diagnosis, for on line (food production line) or at site (animal farms or slaughters, food product packing station *etc.*) rapid detection and identification of food-borne pathogens.

ACKNOWLEDGEMENTS

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