

AN EIGHT-CHAMBER LAB-ON-A-CHIP DEVICE FOR MULTIPLE DETECTION OF *Campylobacter spp* DIRECTLY FROM FECES

Jonas Høgberg¹, Sun Yi², Anders Wolff² and Dang Duong Bang³

¹DTU-Vet, Technical University of Denmark, DENMARK

²DTU-nanotech, Technical University of Denmark, DENMARK

³DTU-Food, Technical University of Denmark, DENMARK

ABSTRACT

In this study, we describe a lab-on-a-chip (LOC) device for detection of Campylobacteriosis - a most common food-borne disease that is caused by *Campylobacter* bacterial infection. By integrating all the steps sample preparation, DNA purification, and PCR amplification on a microchip which can handle eight samples at one time we greatly reduces the detection time in comparison to detection by culture or by conventional PCR.

KEYWORDS: Campylobacter; food-borne pathogens; Lab-on-a-chip; PCR; fecal sample; rapid detection.

INTRODUCTION

Campylobacteriosis is the most common food-borne disease worldwide [1]. It is estimated at least 9 million human campylobacteriosis cases per year in the EU countries [2]. In the USA, for example, two to four million campylobacteriosis cases are reported yearly and in 2006 campylobacteriosis caused 150,000 clinical visits; 13,174 hospitalized and 124 deaths [3]. In the EU, with 175,561 and 160,649 reported cases in 2008 for campylobacteriosis and salmonellosis, respectively, they were the most common infection among the nine most prevalent infection diseases in the EU [4]. In Denmark, despite more than a decade with programs to prevent campylobacter infection both in broiler flocks and human, a total of 3,868 human campylobacteriosis cases were confirmed in 2008 corresponding to 71 per 100.000 inhabitants, making it the most common human diarrhoeal illness [5]. Campylobacter infection has considerably affected society in term of morbidity, health care cost and loss of productivities. In order to comply to the demands from consumers for safe food, free of pathogens, there is an urgent need for development of rapid methods for detection of such pathogens since conventional methods (such as those suggested by the Nordic Committee on Food Analysis) and molecular nucleic acid - based methods for detection and identification of campylobacter infection are time consuming, and laborious [6]. In addition, these methods need special equipments, trained technical staffs and costly. Therefore, a rapid and reliable method for detecting of Campylobacter is urgent needed. We describe here for the first time a lab-on-a-chip (LOC) device for multiple detection of Campylobacter directly from feces. The microchip, which can handle eight samples at one time, integrates all the steps from sample preparation and DNA purification to PCR amplification on an eight-chamber chip. The use of the LOC system reduces the detection time from 5 days by culture or one day by conventional PCR to less than 20 minutes

EXPERIMENTS

Design of the LOC system

The LOC system consists of a disposable microfluidic COP chip with 8-chambers (76 mm/length/ x 26 mm/width/ x 1 mm/height/) fabricating by injection molding; a reusable actuation unit with magnets, heaters for sample preparation and PCR amplification and a 8 channels peristaltic micropump for sample processing. The pump is connected with the outlets of the 8 chambers for addressing all the steps from sample processing, washing, to DNA purification in an automatic way. A picture of the LOC system is show in Figure 1.

On the LOC system sample preparation

Fecal sample 1g chicken feces was collected and suspended in 1 ml of sterile water by vortex. Ten cotton swabs were deepen into the fecal suspension and brought to a 10 ml plastic tube containing 3 ml of sterile water. The swabs were incubated for 5 min at room temperature to release the fecal material. This fecal

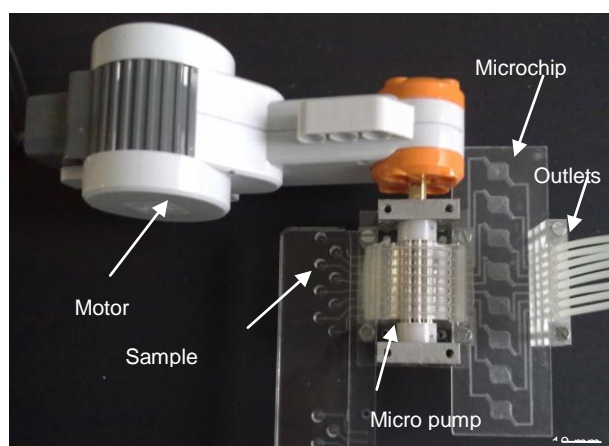
suspension mixture was used to prepare campylobacter spiked samples for testing and validating the performance of the LOC.

DNA samples bacterial strain used in this study was *Campylobacter jejuni* reference strain NTCC-11284 from a bacterial collection at the National Veterinary Institute, Technical University of Denmark (DTU-Vet Aarhus, Denmark). The campylobacter chromosomal DNA was isolated using QIAamp DNA mini kit (Qiagen, Germany). The DNA concentration was determined by Nano drop (Thermo Scientific, USA).

Primers Three sets of primers were designed for identification of *Campylobacter* spp. and their subtype. The universal *Campylobacter* primers (UC) targeted a 300 bps 16sRNA gene fragment of *Campylobacter* spp., while the hippuricase gene (HipO) primers aimed at a 150 bps *hipO* gene sequence specific for *Campylobacter jejuni* species and the primers specific for *Campylobacter coli* (CC) targeting the 190 bps of *Campylobacter coli* 16rRNA gene. All the primers were synthesized at DNA Technology A/S, Denmark (Aarhus Denmark).

PCR master mixture for multiplex PCR contained 10 μ l of 2x Promega mixture (PCR Master Mix, Promega, USA), with 2mM MgCl₂; 0,4 mg/ μ l BSA; 0.04 U/ μ l FastTaq DNA Polymerase, and 4 μ l of three pairs of primers: the UC primers with final concentration of 160 nM, and the HIP and the CC primers with final concentration of 240 nM. All the reagents of PCR were pumped into the PCR chamber using external pump with flow rate 50 μ l/ min. On chip PCR thermocycler with an initiated denaturation step of 94°C for 5 minutes following 30 cycles of 94°C for 15 sec, 54°C for 8 sec and 72°C for 8 sec was conducted. PCR end-point analysis using 2% agarose gel electrophoresis with TAE buffer or Bioanalyser (Thermofisher, Denmark)

Figure 1. The LOC system consists of an eight-chambers microfluidic biochip made by injection molded on 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 the eight chamber microdevice through an 8 channels peristaltic micropump. A mixture of feces and magnetic beads in a bacterial lysis buffer were pumped in to each chamber at flow rate of 10 μ l per minute at room temperature



RESULTS

On chip sample preparation

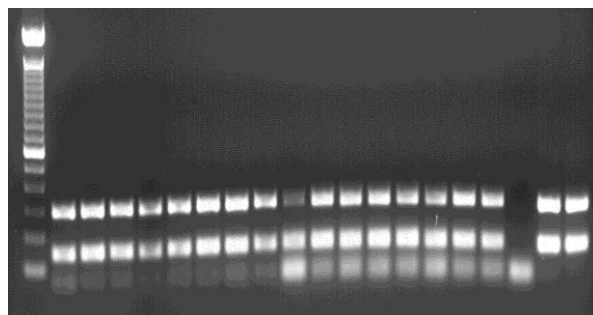
Initiating experiments were conducted to define all the conditions for on chip sample preparation such as the volume of the sample; the ratio of sample and the magnetic bead; the flow rate suitable for sample loading and on chip washing procedure. The best results of these experiments were applied for on chip sample preparation. On chip sample preparation experiments were performed first using purified DNA isolated from campylobacter then with chicken fecal material spiked with campylobacter DNA. The on chip sample preparation is finally performed using 10 μ l mixtures of the fecal material and 5 μ g/ml super paramagnetic beads (Dinabeads Invitrogen, Germany) suspended in lysis buffer. The sample-beads mixture was loaded into each PCR chambers using the pump at a flow rate of 5 μ l /min. After loading the sample, the captured magnetic beads with DNA were washed for 2 minutes using a washing buffer at a flow rate of 500 μ l/min. The magnetic beads were then air dry at the same flow rate for 1 minute at room temperature. After this step the sample is ready for PCR processing. PCR amplification was performed with conditions described above.

On chip PCR amplification

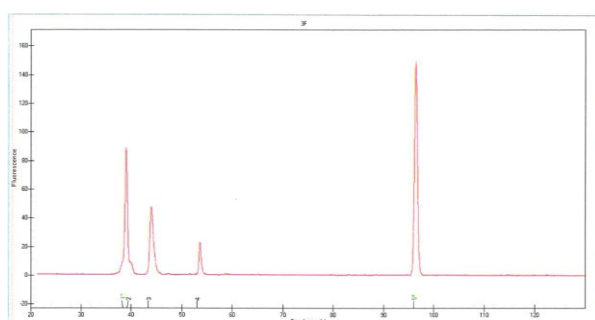
On chip sample preparation and PCR amplification was performed using the 8 chambers LOC system to detect *Campylobacter jejuni* directly from chicken fecal sample. Figure 2 shows the results of a on- chip sample preparation and PCR amplification using the eight-chamber LOC system. The experiment was

performed to detect *Campylobacter jejuni* directly from 16 different *Campylobacter jejuni*-spiked chicken

Figure 2. 2% agarose gel electrophoresis end point analysis for on chip sample preparation and PCR amplification using the eight-chamber Lab-on-a-chip systems. On gel: Lane 1 DNA 100bp molecular weight marker; Lane 2 -17: on chip sample preparation and PCR amplification of the 16 chicken fecal *C. jejuni* spiked samples; Lane 18: Negative control; Lane 19: on chip PCR positive control, lane 20: Conventional on tube PCR positive control with 2ng of DNA from *C. jejuni*.



fecal samples (10^4 CFU/ml of *Campylobacter jejuni*). Strong PCR signals were observed with both the 300 bp of the UC (universal campylobacter) amplicon and the 150 bps of the HipO gene amplicon specific for *Campylobacter jejuni*, confirming the successful of on chip sample preparation and PCR amplification using the eight chamber LOC system to detect *Campylobacter. jejuni* directly from feces. In addition, analysis of one of PCR product of a PCR amplification using the eight-chamber LOC system by Bio-analyzer, a high



Peak State	Peak Number	Mig. Time (secs)	Size (bp)	Corrected Area	Area Ratio	Concentration (ng/μl)	Molarity (nmole/l)	% Total
	2	39.21	19	10.75	0.0978	0.51	39.87	8.17
	3	43.33	56	93.45	0.8500	4.70	127.80	75.93
	4	52.95	151	22.27	0.2026	0.98	9.87	15.89

Sample Peaks Found: 3
 Total Concentration: 6.2 ng/μl
 Total Molarity: 177.5 nmol/l

Figure 3 Result of an analysis of a PCR amplicon using the LOC system by Bioanalyzer (above). High DNA concentration of the amplified PCR product of 4.7ng/μl was archived (right).

DNA concentration of the amplified PCR product of 4.7ng/μl was archived while negative control was negligible.

In conclusion, the newly developed eight-chamber LOC device is a good tool for point of care rapid clinical diagnosis, for rapid screening of food-borne pathogens in industrial animal production (poultry or animal farms and slaughters) or food production line (food manufactures, food product packing stations, Food control units etc.).

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REFERENCES:

- [1] Ziprin et al. Avian Dis. 45, 549-557 (2001).
- [2] Scientific opinions on *Campylobacter* spp. in broiler meat production: EFSA J.9.141 (2011).
- [3] <http://www.cfsan.fda.gov> 2007.
- [4] EFSA journal 2009 130 4-352
- [5] DANMAP 2009 Copenhagen
- [6] Method for detection and enumeration of campylobacter spp. BS.EN ISO 10272-1- (2006).

CONTACT

Dang Duong Bang; ddba@food.dtu.dk