AUTOMATIC FOOD-PATHOGEN DETECTION ON A CENTRIFUGAL MICROFLUIDIC CARTRIDGE IN A COMMERCIALLY AVAILABLE PCR THERMOCYCLER

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ABSTRACT

For the first time facilitated **polymerase chain reaction** (PCR) based detection of **food-pathogens** with a **centrifugal microfluidic cartridge** (GeneSlice) is demonstrated in a field-trial. The ready-to-use GeneSlice [1] comprises 4 reaction chambers with pre-loaded primers and probes for the detection of *Clostridium perfringens*, *Staphylococcus aureus*, *Vibrio cholerae* and *Shigella flexneri* and can be operated in a commercially available real-time PCR thermocycler as a "microfluidic App" [2]. Selectivity of the assays and absence of cross contaminations between wells was confirmed. In a field-trial pathogen spiked enrichment broths from six food-matrices were tested. 14 out of 15 GeneSlices demonstrate successful amplification concordant with manual reference PCR making prior DNA extraction superfluous. Automated analysis of 6 GeneSlices took less than 2 hours with manual preparation time reduced from 30 to 10 minutes.

KEYWORDS: Lab-on-a-Chip, centrifugal microfluidics, food-pathogens, real-time PCR, LabDisk

INTRODUCTION

The World Health Organization addresses the safety of foods as a current threat [3]. Thus detection of pathogens in foods plays an important and indispensable role. The implementation of partly automated food-pathogen assays was focused. The GeneSlice (*Fig. 1*) was adjusted for the simultaneous detection of four common food-pathogens by pre-storing pathogen-specific primers and probes in the reaction chambers. Microfluidic unit operations like metering were automated using centrifugo-thermopneumatic aliquoting [1]. It was possible to process 6 GeneSlice per run (3 samples + 3 controls, *Fig. 2*). The selected pathogens are linked to food poisoning and bioterrorism [4]. The GeneSlice provides a convenient and fast screening platform of different food-matrices for malicious or microbiological contamination.

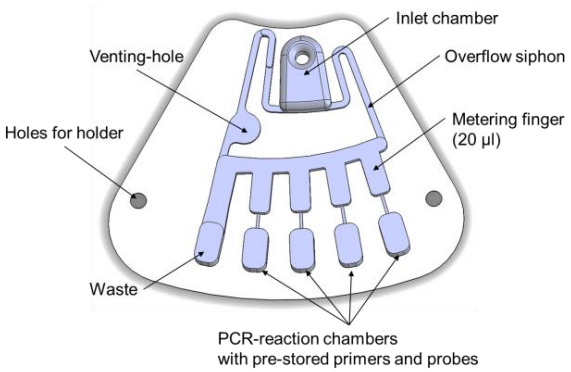

Figure 1: Fluidic layout of food-pathogen GeneSlice. The food-pathogen GeneSlice enables automated centrifuge-thermopneumatic aliquoting [1] of a sample to 4 reaction wells containing pre-loaded primers and probes for the detection of Clostridium perfringens, Staphylococcus aureus, Vibrio cholerae *and* Shigella flexneri. *Successful analysis could be performed with crude lysates of six different food pre-enrichment broths.*

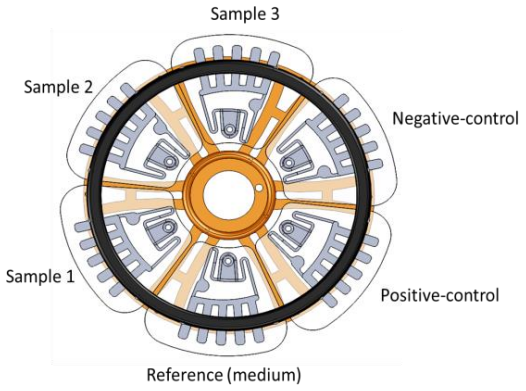

Figure 2: Holder for six GeneSlices: Run configuration. Six GeneSlices can be placed on a holder and processed in one run, including three samples and three controls (no template control (NTC), positive control (PC) and a reference). Amplification and detection of four specific targets in the reaction chambers and readout of the corresponding fluorescence signal during real-time polymerase chain reaction (PCR) leads to results in < 2 h.

MATERIALS AND FUNCTIONAL PRINCIPLE

GeneSlices were fabricated by the HSG-IMIT Lab-on-a-Chip Design- & Foundry Service [5] by micro-thermoforming of thin polymer foils [6]. Pathogen-specific primers and probes were pre-stored in the reaction chambers as follows: Chamber 1: *C. perfringens*, 2: *S. aureus*, 3: *V. cholerae* and 4: *S.flexneri*. Fluidic processing was performed in an off-the-shelf centrifugal thermocycler (Rotorgene Q, Qiagen, Germany) according to [1]. In short: 100 µl of Sample-solution was loaded into the inlet chamber. Due to heating- and cooling steps the liquid was transferred into the aliquoting-structure, ending up with 4 × 20 µl (+ waste) PCR volume per GeneSlice. Subsequent real-time PCR analysis was performed in the same instrument and followed the temperature profile depicted in *Table 1*.

Table 1: Temperature profile of PCR

Hot start of polymerase	5 min @ 95 °C
Cycling 45 ×	15 s @ 95 °C 15 s @ 60 °C 10 s @ 72 °C

For the proof of selectivity 100 µL of sample, a mixture of 50 µL purified DNA (*Table 2*) and 50 µL PCR-Mastermix (2× Rotorgene-Multiplex PCR-Mix, Qiagen, Germany) was loaded into the inlet.

Table 2: Experimental setup for selectivity-testing of GeneSlice; Colors correspond to amplification plots depicted in Fig. 3

GeneSlice No.	**DNA-template**	**Reaction chamber Primer/Probe System**			
		1 *C. perfringens*	**2** *S. aureus*	**3** *S. flexneri*	**4** *V. cholerae*
1 – NTC	-				
2 – PC	*C. perfringens,* 10^4 copies/reaction *S. aureus,* 10^4 copies/reaction *S. flexneri,* 10^4 copies/reaction *V. cholerae,* 10^4 copies/reaction	X	X	X	X
3	*C. perfringens,* 10^4 copies/reaction	X			
4	*S. aureus,* 10^4 copies/reaction		X		
5	*S. flexneri,* 10^4 copies/reaction			X	
6	*V. cholerae,* 10^4 copies/reaction				X

In field-trials food-pathogens were spiked into milk, yoghurt, cheese, minced meat, sausage and poultry. Over-night pre-enrichment cultures of *S. aureus* and *S. flexneri* in CaSo-Bouillon were incubated under aerobic conditions. *Clostridium perfringens* was cultivated in cooked meat medium under anaerobic conditions. For food-matrix tests 1 ml of all enrichment cultures of *S. aureus*, *S. flexneri* and *C. perfringens* were pooled. Bacterial DNA was released by chemical, mechanically assisted lysis (*mericon* DNA Bacteria Plus Kit, Qiagen, Germany). All supernatants were diluted 1:100 in TE-buffer, spiked with DNA of *Vibrio cholerae* and used for PCR. To exclude cross-reactivity with matrices, references, which include only media, have also been tested. To test for false-positive or false-negative results each run contains a no template control (NTC), positive control (purified DNA of all pathogens, PC) and a reference besides the three samples of interest (*Fig. 2*).

RESULTS AND DISCUSSION

Results of real-time PCR in GeneSlices have been compared to manual reference PCR in tubes. Variation in C_q-values was below 2 cycles although the performance and linearity of the PCR in GeneSlices were similar to those from reference PCR. Selectivity of the PCR assays has been confirmed. No well-to-well cross-contaminations were observed (*Fig. 3*).

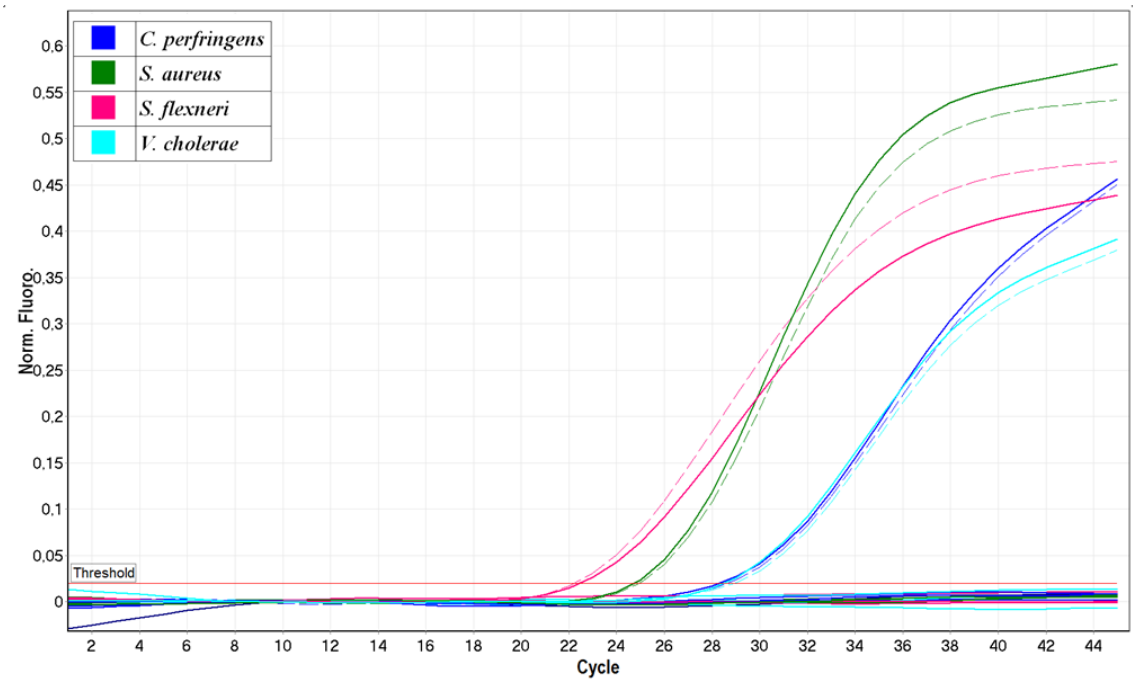

Figure 3: Amplification curves of real-time PCR for proof of selectivity. Selectivity of the assays on the GeneSlice was tested with GeneSlices containing purified target DNA of one of the four pathogens (GeneSlices 3-6, solid curves). To avoid false-negative and -positive results corresponding controls (NTC / PC) of all pathogens were tested in the same run (GeneSlices 1 and 2). Positive controls contained DNA targets of all four pathogens (dashed curves). Real-time PCR resulted in 2 positive amplifications per pathogen (one from positive control and one for detection of pathogen).

Additionally, pathogen-detection on the GeneSlice was demonstrated using lysed food enrichment broth of different milk- and meat products instead of purified DNA as the sample material. 14 out of 15 experiments on the GeneSlice were concordant with manual reference PCR in tubes proving the possibility to detect pathogens directly from a lysate of enrichment cultures and making prior DNA extraction superfluous. By using the GeneSlice, manual preparation time was reduced from about approximately 30 minutes to 10 minutes per sample.

CONCLUSION

A selective, simultaneous detection of four food-pathogens was demonstrated using a centrifugal microfluidic GeneSlice operated in an off-the shelf PCR thermocycler. Six GeneSlices can be processed in parallel in less than two hours, including four GeneSlices for target pathogen detection, one for positive control and one for no-template control. Performance of PCR on GeneSlices was comparable to manual reference PCR (variation < 2 cycles) at equal linearity and efficiency. Additionally, pathogen-detection from crude lysates of enrichment broths, originating from six different food-matrices, was successfully demonstrated in 14 out of 15 experiments. Concurrently manual preparation time is reduced from 30 minutes to 10 minutes.

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