REAL-TIME PCR BASED FOOD PATHOGEN DETECTION ON A CENTRIFUGAL MICROFLUIDIC FOIL DISK INCLUDING POSITIVE- AND NO-TEMPLATE-CONTROLS

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ABSTRACT

We designed and evaluated a novel microfluidic structure for the **real-time PCR** based **detection of six common food pathogens** on a **centrifugal microfluidic foil disk**, including onboard **positive- and no-template-controls** for each of the targets. The microfluidic design enables for geometric multiplexed PCR in a **standard centrifugal real-time PCR thermocycler**. The **limit of detection** was 0.1 pg target DNA what corresponds to **17-56 DNA copies** per PCR reaction.

KEYWORDS: Lab-on-a-Chip, Centrifugal microfluidics, PCR on a chip, Food pathogen testing

INTRODUCTION

When performing nucleic acid based testing, it is mandatory to exclude false negative and false positive results. Whereas in standard laboratory diagnostics, the integration of positive- (PTC) and no-template controls (NTC) is common practice, up to date, lab-on-a-chip demonstrators usually miss such controls. Hence this work is dedicated to the design and evaluation of integrated control systems for lab-on-a-chip based real-time PCR assays demonstrated by the nucleic acid based detection of six common food pathogens. While PTC's usually serve as inhibition control and validate the functionality of set-up and reagents (false negatives), NTC's are conducted to scan for unwanted DNA contaminations (false positives).

MATERIALS AND METHODS

A schematic of the developed microfluidic structure is depicted in Fig. 1. Foil disks (COP ZF 14 foil, Zeon chemicals, thickness 188 µm, diameter 130 mm) were manufactured by a softlithographic replication process [1]. To enhance priming, capillary siphons were coated with 5 µL of a hydrophilic agent (Vistex 111-50, Filmspecialities Inc.) dissolved in isopropanol (conc. 2% v/v). Hydrophobic valves were coated with 0.25 µL of an amorphous fluoropolymer (Teflon AF 1600, DuPont) dissolved in a fully fluorinated liquid (Fluorinert FC77, 3M) (conc. 0.5% w/w). One set of specific primers (10 µM, 0.3 µL each) and TaqMan probe (10 µM, 0.2 µL) for PCR was stored in each detection- and positive-control cavity by airdrying. Additionally, the cavities for positive controls were loaded with 0.1 pg and 1 pg of the corresponding pathogen DNA respectively, while the no-template control cavity was loaded with primers and probes for all target organisms but no DNA. After microfluidic processing, the disk was placed in a commercial real-time PCR thermocycler (Rotorgene 2000, formerly Corbett Research now Qiagen) for amplification and readout (Fig. 2) [1].

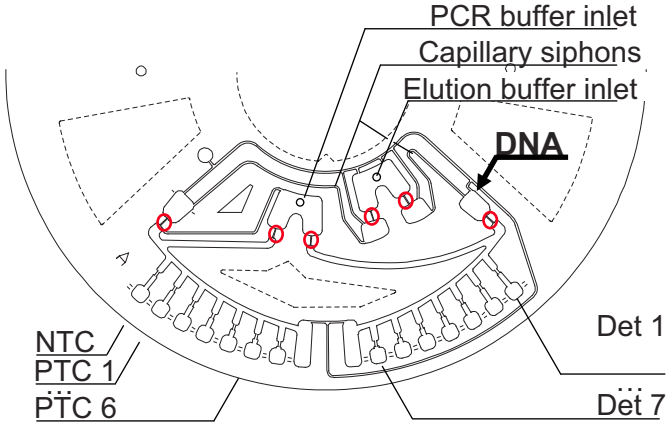

Fig 1: Fluidic design of the foil disk. Amplification / detection takes place in fluidically separated cavities: target detection (Det 1 – 7), positive control (PTC 1 – 6), no-template control NTC. Red circles mark hydrophobic valves .

Fig 2: Microfluidic foil disk for food pathogen detection placed in the real-time PCR thermocycler (Rotorgene 2000, formerly Corbett Research; now Qiagen).

EXPERIMENTAL

At the beginning, the disk is mounted onto a centrifugal test set-up and 100 µL of elution buffer (DI-water) and 100 µL of PCR buffer (Light Cycler 480 Probes Master, Roche Applied Sciences) are pipetted into the corresponding inlets. The disk is then accelerated to a rotational frequency of 8 Hz (5 sec.) leading to an equal distribution of elution- and PCR buffer in the respective inlet chambers (Fig. 3A). Subsequent rotation at 15 Hz (10 sec) aliquots the PCR buffer into 5 µL subvolumes. A detailed description of this microfluidic aliquoting process is given in [3]. Frequency alternation between 7 Hz and 25 Hz (10 times) then transfers the PCR buffer into the amplification cavities where the prestored, dried primers and probes are automatically rehydrated (Fig. 3B). Rotation is stopped and the capillary siphons prime with elution buffer due to capillary forces. The DNA sample is loaded into the DNA elution chamber which will in a prospective design be the elution chamber of an integrated DNA-extraction module [2]. Reacceleration to 8 Hz (100 sec.) gates the elution buffer into the DNA elution chamber (Fig. 3C). Rotation at 18 Hz (30 sec) splits the elution buffer into 5 µL volumes that are then transferred into the PCR cavities during frequency alternation between 5 - 25 Hz (20 times) (Fig. 3D). Total fluidic processing requires about 7 minutes. Subsequently, the disk is transferred from the centrifugal test set-up into the real-time PCR instrument. Thermocycling is started with an initial hot start (95°C / 7min) followed by 50 cycles of denaturation (95°C / 15 sec.), annealing (60°C / 20 sec.) and extension (72°C / 30 sec.).

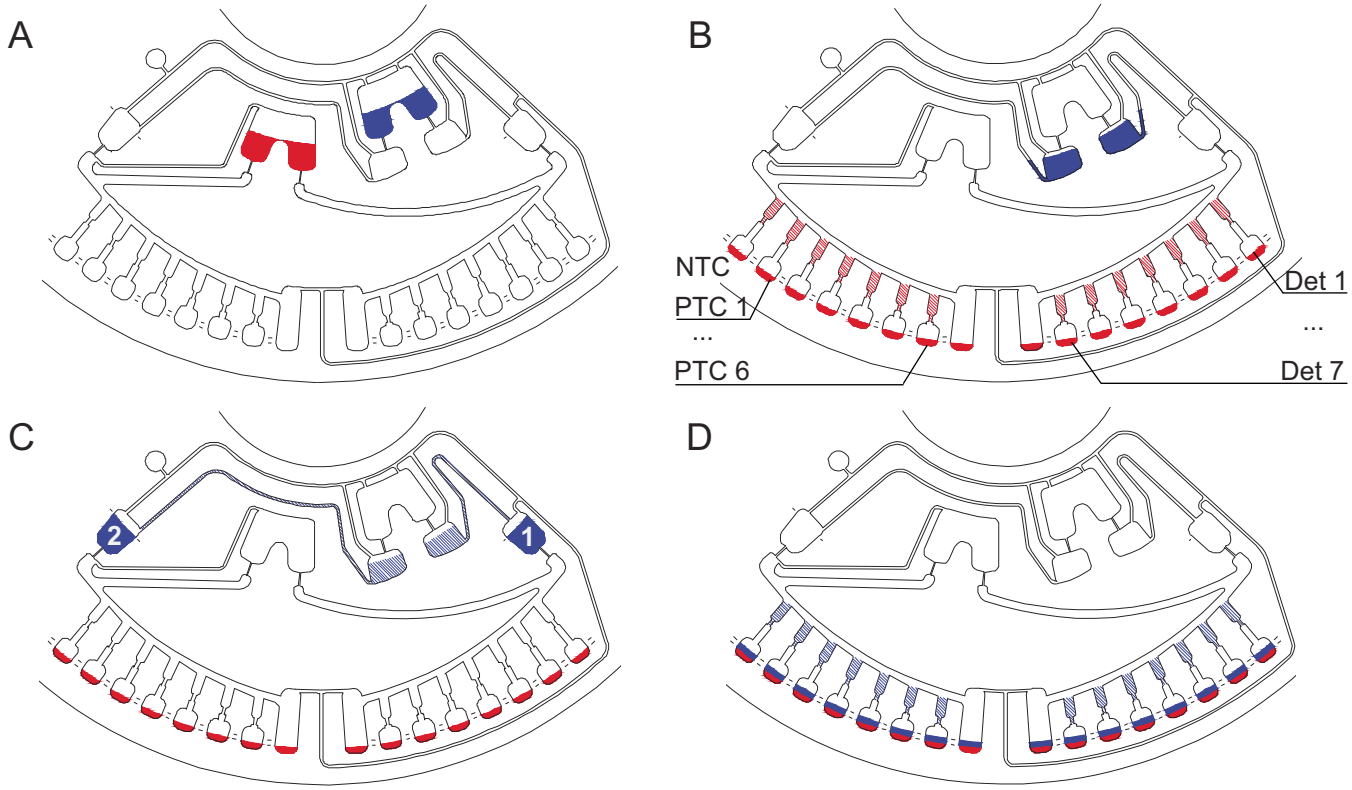

Fig. 3: Elution buffer (blue) and PCR buffer (red) are loaded into the inlets (A). Acceleration to 8 Hz (5 sec.) and 15 Hz (10 sec.) splits PCR buffer into aliquots of 5 µL. Frequency alternation transfers PCR buffer into the amplification cavities Det 1-7, PTC 1-6 and NTC (B). The disk is stopped and the capillary siphons prime with elution buffer. The DNA sample is loaded into the DNA elution chamber. Reacceleration to 8 Hz (100 sec.) then gates one portion of the elution buffer into the DNA elution chamber (1) while the second portion of the elution buffer is gated into a dummy chamber with equal size (2) which was integrated for fluidic symmetry only (C). Rotation at 18 Hz splits elution buffer into 5 µL volumes that are then transferred into the PCR cavities (D). Now, thermocycling can be started.

RESULTS AND DISCUSSION

On disk amplification was successfully demonstrated for 1 pg and 0.1 pg DNA per PCR reaction corresponding to 170 - 560 DNA copies (Fig. 4) and 17 – 56 copies (Fig. 5) respectively. In detail: *Campylobacter jejuni* (560 copies and 56 copies), *Citrobacter freundii* (180 cop. and 18 cop.), enterohemorrhagic *Escherichia coli* "EHEC" (170 cop. and 17 cop.), *Listeria monocytogenes* (310 cop. and 31 cop.), *Salmonella typhimurium* (180 cop. and 18 cop.) and *Staphylococcus aureus* (320 cop. and 32 cop.) were successfully amplified and detected. Onboard no-template control could detect contaminations of 1.7 – 5.6 DNA copies after 50 cycles (except *Citrobacter*: limit of detection was 18 cop.) (Data not shown).

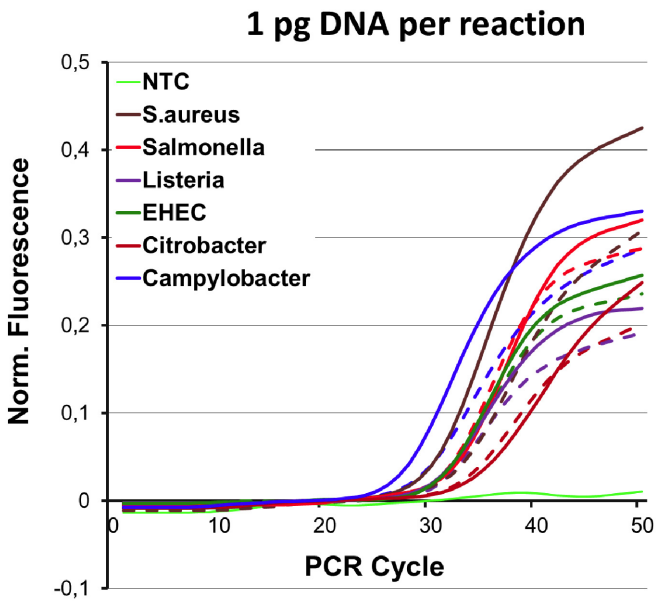

Fig 4: Amplification plot for on-chip amplification of 1 pg genomic DNA per food pathogen per amplification chamber. Continuous lines represent PCR results from detection cavities while dashed lines represent PCR results from positive controls with 1 pg prestored DNA.

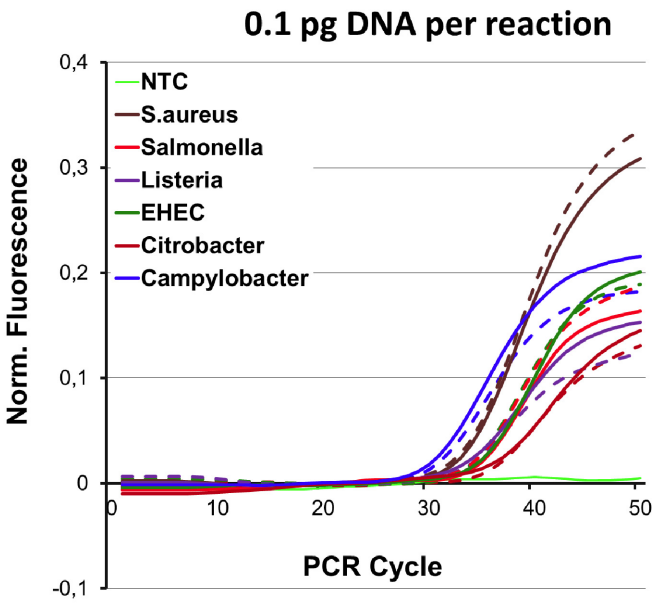

Fig 5: Amplification plot for on-chip amplification of 0.1 pg genomic DNA per food pathogen per amplification chamber. Continuous lines represent PCR results from detection cavities while dashed lines represent PCR results from positive controls with 0.1 pg prestored DNA.

CONCLUSION AND OUTLOOK

We successfully demonstrated a centrifugal microfluidic lab-on-a-chip cartridge for the sensitive real-time PCR based detection of six common food pathogens using a standard laboratory real-time thermocycler. Artificial DNA contaminations could successfully be detected by onboard no-template controls while additional positive controls ensured amplification and detection functionality of each PCR system. The microfluidic chambers are arranged in a way that a connection to an upstream DNA extraction module (published in [2]) might be possible, paving the way for complete sample-to-answer analysis on a chip. Integrated prestorage of liquid reagents [4, 5] would further facilitate the operation.

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