DNA BASED SAMPLE-TO-ANSWER DETECTION OF BACTERIAL PATHOGENS ON A CENTRIFUGAL MICROFLUIDIC FOIL CARTRIDGE

O. Strohmeier[1], B. Kanat[1], D. Bär[2], P. Patel[3], J. Drexler[4], M. Weidmann[5], T. van Oordt[1], G. Roth[1,2], D. Mark[1], R. Zengerle[1,2] and F. von Stetten[1,2]

[1]HSG-IMIT, Wilhelm-Schickard Straße 10, 78052 Villingen-Schwenningen, GERMANY[1]
[2]Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, GERMANY
[3] Robert Koch Institute, Centre for Biological Security, German
[4]QIAGEN Lake Constance, Germany
[5] Department of Virology, University Medical Center Göttingen, Göttingen, Germany

ABSTRACT

An **integrated** and **portable centrifugal microfluidic *LabDisk*** system for **DNA based sample-to-answer detection of bacterial pathogens** is presented. For the first time, **DNA extraction** and subsequent **multiplexed isothermal real-time amplification** by recombinase polymerase amplification (RPA) are integrated on one centrifugal microfluidic chip. *Bacillus anthracis* and *Francisella tularensis* were successfully detected from blood plasma with **time-to-result < 45 minutes**. In future, the *LabDisk* system will be applicable for simultaneous, multiplexed pathogen detection of RNA and DNA targets.

KEYWORDS: Lab-on-a-Chip, Centrifugal Microfluidics, LabDisk, RPA

INTRODUCTION

Automation and miniaturisation of molecular diagnostic testing with sample-to-answer capability is expected to have high impact on point-of-care diagnostics [1, 2]. By microfluidic integration of all process steps, untrained users would be able to conduct complex medical testing what is a prerequisite, e.g. for field testing or to improve medical care in the third world. We present a centrifugal microfluidic *LabDisk* system for the fast detection of two pathogens relevant in biological warfare.

MATERIALS AND METHODS

All *LabDisks* were fabricated by micro-thermoforming from 188 µm COP foils (COP ZF 14, Zeon Chemicals, USA) [3]. Siphons S_2 and S_3 were hydrophilically coated with 0.2 µL 1:20 Vistex-isopropanol (Vistex 111-50, Filmspecialities Inc., USA) while the DNA extraction area was hydrophobically coated with 2 x 100 µL 0.5% w/w Teflon AF (DuPont, USA) in Fluorinert FC77 (3M, Belgium) (Figure 1). Reverse and forward primers (0.42 µL 10 µM each) and FAM labeled probe (0.12 µL 10 µM) for specific amplification of *B. anthracis* are prestored in amplification chambers 2-5 and for *F. tularensis* in chambers 6-8. RPA lyophilisate pellets, containing enzymes for isothermal amplification by RPA (Twist Amp exo, Twist Dx, UK), are placed in the mixing chamber and the cartridge is sealed with a pressure sensitive adhesive foil (#900 320 HJ Bioanalytic, Germany).

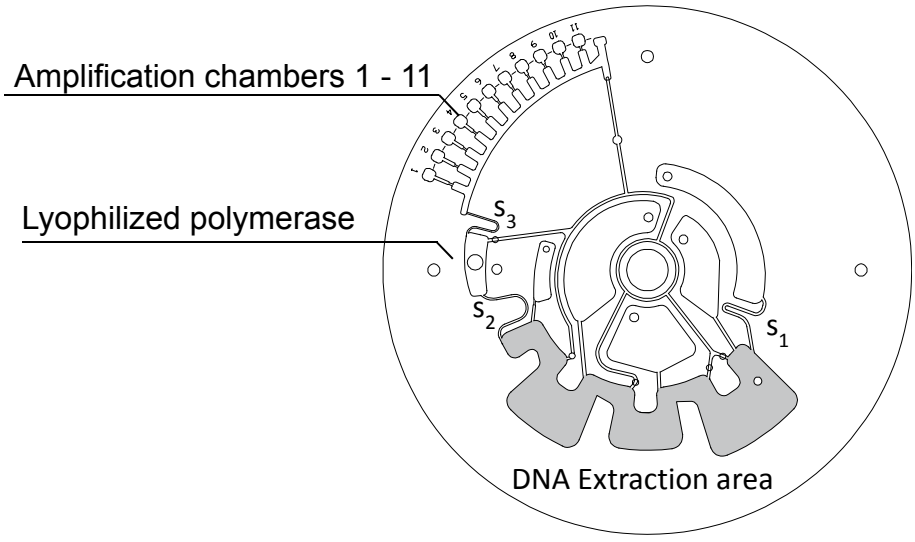

Figure 1: Microfluidic layout of a LabDisk. Capillary siphons S2 and S3 are coated with Vistex-isopropanol, DNA extraction area (grey shaded) was coated with Teflon AF. Specific primers and probes were prestored in defined amplification chambers. Lyophilized polymerase pellet was placed into the mixing chamber.

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EXPERIMENTAL

The sample (200µL blood plasma spiked with inactivated *B. anthracis* and *F. tularensis*, magnetic beads and Proteinase K) is mixed directly before processing and then pipetted into the lysis chamber. Binding buffer (450 µL), lysis buffer (300 µL) and two washing buffers (500 µL each) (Instant MP extraction Kit, Analytik Jena, Germany) are loaded into the corresponding inlets and automatic processing is started as described in Figure 2. After lysis, DNA is extracted by magnetic beads via an automated bead transport method described in [4]. The method solely requires a spinning device equipped with a locally fixed permanent magnet and a programmable frequency protocol. When starting the protocol magnetic beads are transported through binding buffer, washing buffer finally into a chamber that contains the elution buffer. The elution buffer has to be manually added during a predefined stop in rotation. Then the automated protocol continues and the eluate is mixed with lyophilized RPA reagents. Finally, the resulting mix is aliquoted into 10 amplification chambers containing prestored primers and probes. Excess liquid is gated into chamber 11. Specific isothermal DNA amplification by RPA starts by rehydration of primers and probes and real-time fluorescence signals are recorded.

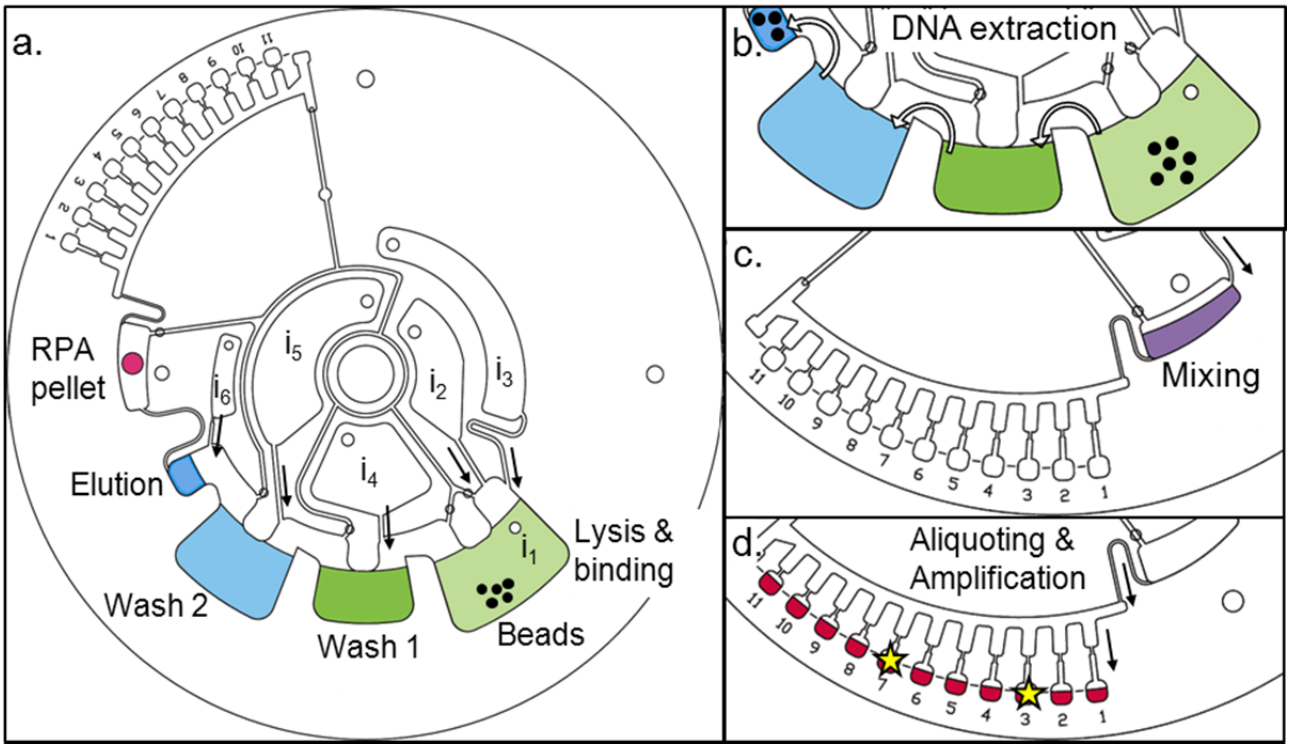

Figure 2: Microfluidic process-flow. (a) Sample with magnetic beads, lysis-, binding- and two washing buffers are pipetted into inlets i_1, i_2, i_3, i_4 and i_5 respectively. After rotation is started, all reagents are gated radially outwards by centrifugal forces. (b) After lysis, the beads bind DNA and are transported through the washing buffers into the elution chamber by magnetic actuation [4]. Elution buffer is manually added during a pause in the frequency protocol. (c) The eluate is then gated into a chamber with prestored RPA lyophilisate pellets. After rehydration and mixing, the mastermix is aliquoted into 10 µL volumes and gated into chambers (1 – 10) where amplification starts immediately after the prestored primers and probes are rehydrated (d). Cavity 11 was used as waste chamber.

RESULTS AND DISCUSSION

For demonstration of the automated sample-to-answer workflow, 6 x 10^4 genome equivalents of *B. anthracis* and 6 x 10^6 genome equivalents of *F. tularensis* were successfully detected in less than 45 minutes (~35 min. lysis and DNA extraction; ~10 min. amplification) (Figure 3). Processing and detection were performed by the *LabDisk* Player (QIAGEN Lake Constance, Stockach, Germany), a portable device with a size of approximately 18 x 28 x 15 cm³ and a weight of approximately 2kg that features FAM fluorescence detection, adjustable temperature up to 60 °C and the possibility to run predefined frequency protocols (Figure 4).

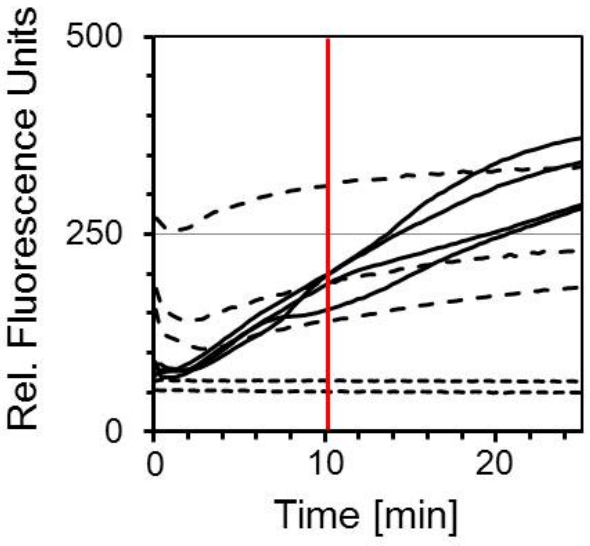

Figure 3: Amplification plot showing successful amplification of B. anthracis (solid line) detected in chambers 2-5 and F. tularensis (dashed line) in chambers 6-8 below 10 minutes (red vertical line). Cavities without prestored primers (1, 9, 10) don't show any increase in fluorescence (dotted line). Before amplification starts, fluidic processing for DNA extraction requires about 35 minutes.

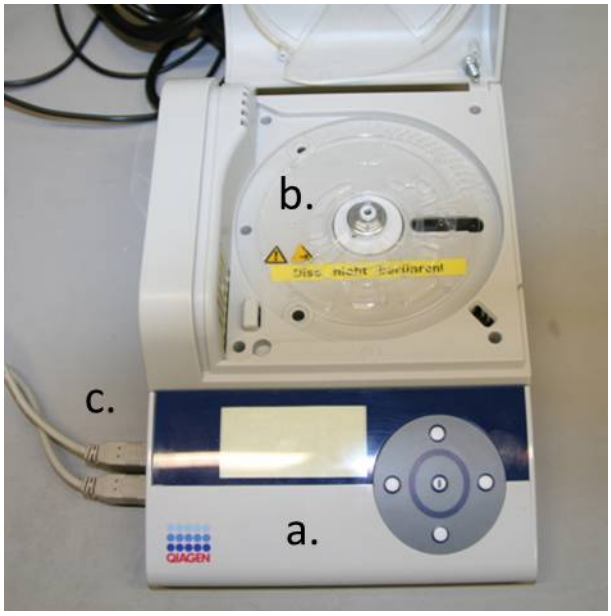

Figure 4: LabDisk player (a) with LabDisk (b) and connection to notebook (c). The LabDisk player was developed by QIAGEN Lake Constance.

CONCLUSION AND OUTLOOK

We demonstrated rapid sample-to-answer detection of two different pathogens on DNA level. In future, eleven separate amplification chambers will allow for panel testing of different pathogens. Further developments will focus on the replacement of the manual addition of elution buffer. Prestorage of liquid reagents [5] would further allow for self-sufficient processing. Due to the low weight of the *LabDisk* Player and the monolithic cartridge design, the system ideally meets the requirements for an application at the point-of need where a high degree in automation is required.

ACKNOWLEDGEMENTS

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CONTACT

*O. Strohmeier; Phone: +49 761 / 203-73232; Mail: oliver.strohmeier@hsg-imit.de