FULLY INTEGRATED PCR DETECTION OF PATHOGENS FOR FAST DIAGNOSIS OF NEONATAL SEPSIS ON LABDISK

G. Czilwik[1*], T. Messinger[1], O. Strohmeier[1], F. von Stetten[1,2], R. Zengerle[1,2,3], P. Saarinen[4], J. Niittymäki[4], K. McAllister[5], O.Sheils[5], J. Drexler[6] and D. Mark[1]

[1] HSG-IMIT, Freiburg, Germany
[2] IMTEK - University of Freiburg, Germany
[3] BIOSS – Centre for Biological Signalling Studies, University of Freiburg, Germany
[4] Mobidiag Ltd, Espoo, Finland
[5] Trinity College Dublin, Ireland
[6] Qiagen Lake Constance GmbH, Germany

ABSTRACT

We developed a fully automated centrifugal microfluidic `LabDisk` system for highly sensitive detection of neonatal sepsis pathogens in human serum samples within 4 hours. All necessary assay steps are integrated on a single test carrier: DNA extraction, pre-amplification and geometrically multiplexed realtime PCR. Processing solely requires loading of the sample and extraction reagents. Afterwards the entire process is automated using a rotational protocol in a mobile processing device. We demonstrate detection down to 3 colony-forming-units (cfu) of *Staphylococcus warneri,* 150 cfu of *Streptococcus agalactiae,* 5 cfu of *Escherichia coli* and 18 cfu of *Haemophilus influenzae* in 200 µL of serum.

KEYWORDS: Centrifugal Microfluidics, Lab-on-a-Chip, Pathogen Detection

INTRODUCTION

Neonatal sepsis remains among the major causes of infant death worldwide [1] and management of patient sepsis is challenged by short-comings in current diagnostics. The diagnostic gold standard is blood culture requiring 1-3 days for a conclusive result. In particular for neonatal sepsis, where disease progression can be extremely fast, a rapid diagnostic tool for identification of the causative pathogen is thus of major interest. In this context, we developed a rapid and fully automated LabDisk system that could reduce reporting hold-ups and help to give clinicians facts-based treatment options as early as possible.

EXPERIMENTAL

All experiments were conducted using LabDisks of COP (COP ZF 14, Zeon Chemicals, USA) that were fabricated by micro-thermoforming [2]. The LabDisks were previously equipped with all necessary reagents for the preamplification and realtime PCR amplification: Specific oligonucleotides, dry PCR reagent beads (illustra RTG, GE Healthcare) and a rehydration buffer prestored in a stick-pack [3]. The LabDisk was sealed with an adhesive foil (HJ Bioanalytic, Germany). Processing was conducted in a prototype LabDisk player (Figure 1a, Qiagen Lake Constance, Germany). For characterization tests of our system, we cultivated bacterial pathogens *Staphylococcus warneri, Streptococcus agalactiae, Escherichia coli* and *Haemophilus influenzae* each overnight. The bacterial concentration was estimated the next day using optical density measurements at a wavelength of 600 nm, which was later on confirmed by Koch colony counting. Thereafter, 200 µL of human serum were each spiked with known concentrations of the pathogens to obtain model samples, which were then analyzed as follows: 200 µL of the model samples and 20 µL of magnetic beads were pipetted into sample inlet chamber (i1, Figure 1b). Lysis buffer (600 µL), binding buffer (450 µL), two ethanol-based washing buffers (495 µL each) and 100 µL of elution buffer (all from Instant MP extraction Kit, Analytik Jena GmbH, Germany) were loaded into the corresponding inlets (i2-i6, Figure 1b). The automated fluidic process-flow was then started. No additional hands-on time was required afterwards. The model samples were in parallel analyzed with a manual reference method that uses identical DNA extraction and PCR reagents in standard reaction tubes. The PCRs were thereby conducted in a commercial thermo-cycler.

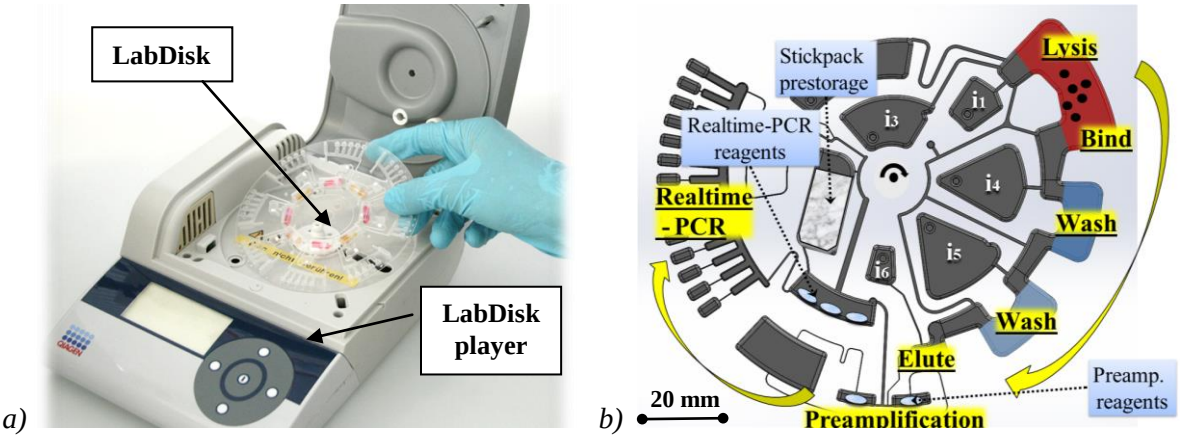

Figure 1: (a) The LabDisk platform with a portable point-of-care processing device. The LabDisk is manually mounted on a rotating axis. The processing device conducts a predefined rotational protocol, controls temperature and performs optical readout for automation of all necessary assay and liquid handling steps. (b) LabDisk design with integrated structures for automated DNA extraction (Lysis, DNA binding, washing and elution), PCR-preamplification and 2nd realtime PCR.

RESULTS AND DISCUSSION

We were able to demonstrate full automation of the complete assay protocol on a single test carrier. The integrated LabDisk assay showed detection of Gram+ bacteria *Staphylococcus warneri* and *Streptococcus agalactiae* down to 3 colony forming units (cfu) and 150 cfu in the sample, respectively. For Gram- bacteria *Escherichia coli* and *Haemophilus influenzae* down to 5 cfu and 18 cfu were detected in the sample, respectively. For control samples (pure human serum) no detection signal was generated for all bacterial targets. The results perfectly matched to the manual reference in terms of assay sensitivity. The LabDisk assay had a process time of approximately 3 hours and 50 minutes, which would provide clinicians with vital information within hours instead of days, as for blood culture.

CONCLUSION

We successfully integrated DNA extraction, preamplification and realtime PCR for specific diagnosis of neonatal sepsis on a single test carrier. The LabDisk assay enables fast detection of pathogens in human serum. Follow-up development will include pre-storage of all liquid buffers to further decrease manual handling. The analysis time could very likely be further reduced by faster heating and cooling rates of the LabDisk player and by an optimization of the PCR thermocycling parameters towards shorter holding times. Our LabDisk assay principle could also be adapted to other diagnostics panels (e.g. respiratory diseases) or to resistance marker testing.

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CONTACT

*G. Czilwik, Tel: +49-761-20373231; gregor.czilwik@hsg-imit.de