# An Integrated Centrifugal Lab-on-a-Chip System for fully automated detection of pathogens via Real-time PCR

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## Summary

We present an **integrated** and **portable** centrifugal microfluidic foildisk ("LabDisk") for **DNA based sample-to-answer detection** of bacterial pathogens featuring:

- Magnetic bead-based **DNA extraction**
- Real-time **Polymerase-Chain-Reaction (PCR)**
- Automated process, **time-to-result ~ 3 hours**
- Detection of ***Staphylococcus aureus*** as a model study
- **Prestorage of PCR reagents**

For processing of the LabDisk, the small and portable LabDisk-Player was used, which can be applied at the point-of-care.

## Motivation

For many infectious diseases, such as sepsis, there is a growing demand for fast, small and easy-to-use diagnostic tests. Fully automated Lab-on-a-Disk systems combine portability with fast turnaround times. This enables untrained users to conduct complex medical tests and to decrease reporting hold-ups in medical practice. We implemented a fast point-of-care sepsis test for detection of pathogens on a our centrifugal microfluidic LabDisk platform. The system has the potential to enable proper use of correct antibiotics and may help to decrease the spread of antibiotic resistances in sepsis treatment.

## Materials and Methods

The LabDisk was fabricated by micro-thermoforming of COP foils. Complete pre-storage of PCR reagents comprised air-dried primers, FAM labeled probes and lyophilized PCR beads (illustra mix RTG, GE Healthcare) in the reaction chambers. The surface of the DNA extraction module was hydrophobically coated using Teflon AF to prevent adsorption of magnetic particles. The foildisk was finally sealed with a pressure sensitive adhesive foil. The tests were performed in the "LabDisk Player" (Fig.2), a portable device with a weight of ~ 3 kg that features fluorescence detection, PCR-thermocycling and the possibility to run predefined frequency protocols.

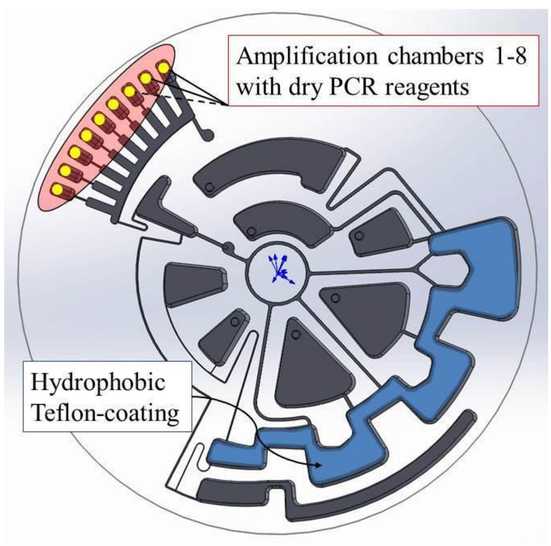


Fig.1 – Integrated LabDisk for pathogen detection.


Fig.2 – LabDisk Player – A centrifugal processing device with integrated PCR-thermocycling chamber

## Fluidic process flow

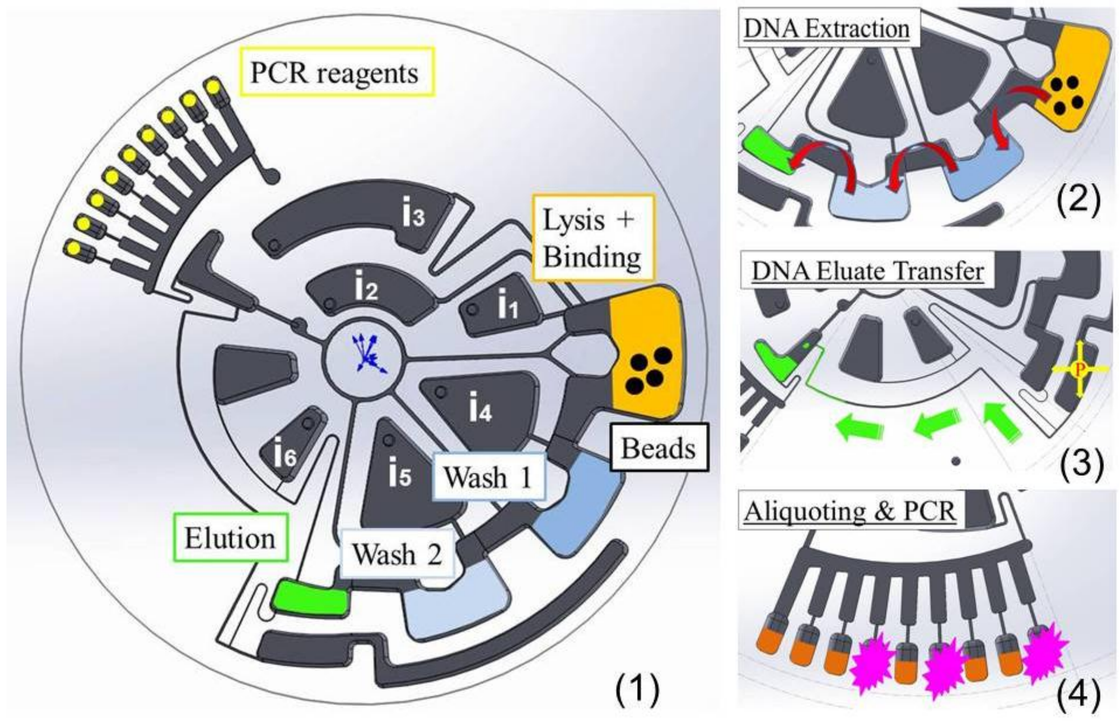


Fig.3 - Fluidic process-flow: (1) Sample with magnetic beads, lysis-, binding-, elution- and washing buffers are pipetted into inlets $i_1$-$i_6$. Then the automated protocol starts: Reagents are pumped radially outwards by centrifugation. (2) Cells are lysed, beads bind the DNA and are transported through the washing buffers into the elution buffer by magnetic actuation. (3) The eluate is pneumatically pumped radially inwards, aliquoted into 20 µL volumes, and transferred into the reaction chambers. Lyophilized PCR beads are rehydrated and thermocycling with fluorescent real-time readout is performed (4).

## Results

For demonstration of the automated sample-to-answer workflow, 1.6 x $10^6$ genome equivalents of ***S. aureus*** were successfully detected in approximately 3 hours (~35 min. lysis and DNA extraction; 2 h 30 min. amplification by thermocycling) (Fig. 4).

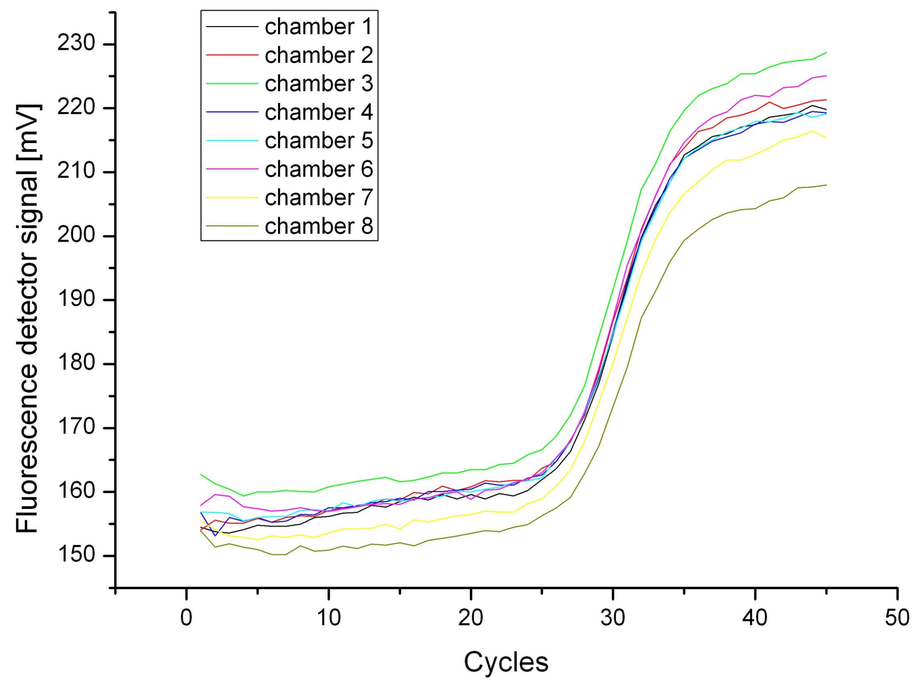


Fig.4 – Positive amplification signal for *S.aureus* in LabDisk Player

## Conclusions

We demonstrated rapid sample-to-answer detection of *S.aureus* as a proof-of-principle. The low dimensions of the LabDisk Player and the monolithic foildisk design ideally meet the requirements for an application at the point-of-care. The parallel detection of a full pathogenic panel in multiple reaction cavities as well as prestorage of liquid reagents is currently under development. Acceleration of thermocycling needs to be addressed.

## Partners

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## Acknowledgements

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