Automated and miniaturized detection of biological threats with a centrifugal microfluidic system

D. Mark*[a], T. van Oordt[a], O. Strohmeier[a], G. Roth[b], J. Drexler[d], M. Eberhard[d], M. Niedrig[e], P. Patel[e], A. Zgaga-Griesz[f], W. Bessler[f], M. Weidmann[g], F. Hufert[g], R. Zengerle[a,b,c] and F. von Stetten[a,b]

[a]HSG-IMIT - Institut für Mikrotechnik und Informationstechnik, Wilhelm-Schickard-Strasse 10, 78052 Villingen-Schwenningen, Germany

[b]Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, Germany.

[c]Centre for Biological Signalling Studies - BIOSS, Universiy of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, Germany

[d]QIAGEN Lake Constance GmbH, Jacques-Schiesser-Strasse 3, 78333 Stockach, Germany

[e]Robert Koch Institut, Nordufer 20, 13353 Berlin, Germany

[f]University Medical Center Freiburg, Institute for Molecular Medicine and Cell Research, Stefan-Meier-Str. 8, 79106 Freiburg, Germany

[g]University Medical Center Göttingen, Department of Virology, Kreuzbergring 57, 37075 Göttingen, Germany

ABSTRACT

The world's growing mobility, mass tourism, and the threat of terrorism increase the risk of the fast spread of infectious microorganisms and toxins. Today's procedures for pathogen detection involve complex stationary devices, and are often too time consuming for a rapid and effective response. Therefore a robust and mobile diagnostic system is required. We present a microstructured LabDisk which performs complex biochemical analyses together with a mobile centrifugal microfluidic device which processes the LabDisk. This portable system will allow fully automated and rapid detection of biological threats at the point-of-need.

Keywords: Lab-on-a-chip, point-of-need, microfluidics, pathogen detection, genotyping, PCR, immunoassays

1. THE LABDISK: AN INTEGRATED ANALYSIS INSTRUMENT FOR THE POINT OF NEED

1.1 Overview

The LabDisk platform enables automated and integrated analyses and diagnostics by microfluidic liquid control. One essential component is a disposable test carrier in the shape of a microstructured polymer disk. It is processed by a mobile, robust centrifugal processing device which mainly consists of a rotational motor, an optical detector and a heating unit (Figure 1). As reported previously[1], the system is designed for point-of-need applications: the ready-to-use disposable can be equipped with pre-stored reagents. Demonstrators for nucleic acid based detection of pathogens[2,3] have been reported previously and the panel of applications is currently expanded towards immunoassays. This unique design enables fast (< 1 hour), accurate diagnostics with a portable, easy to operate and robust system.

*daniel.mark@hsg-imit.de; phone +49 761 203-73246; fax +49 761 203-73299; www.loac-hsg-imit.de

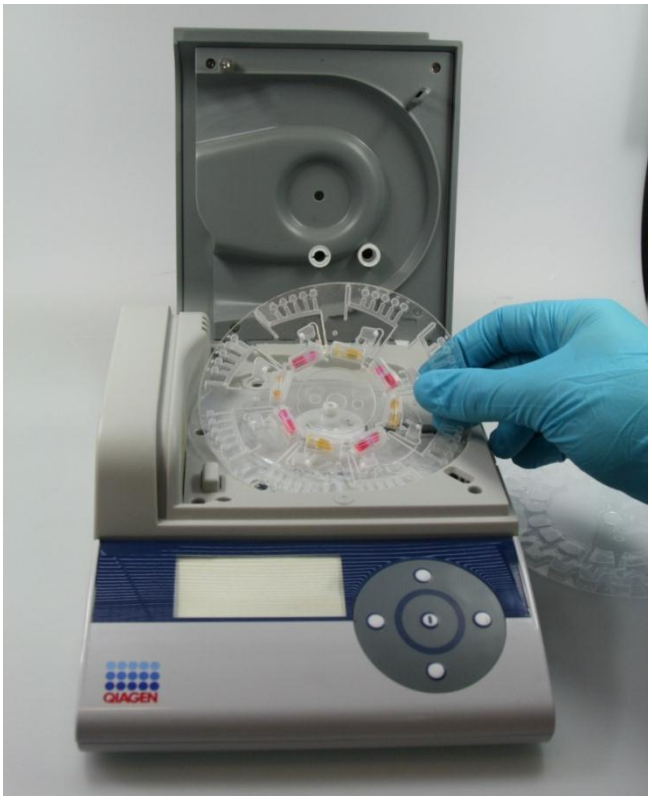

Figure 1: Portable diagnostic system for point-of-need applications. It enables nucleic acid based detection of pathogenic microorganisms and the immunoassay based detection of toxins. Shown is a disposable for genotyping of bacteria by isothermal amplification and detection (polymer disk in hand) as well as the centrifugal processing device.

1.2 The disposable test carriers

The disposables are microstructured polymer disks which include all of the required liquid handling operations to perform biochemical analysis. The foil based production approach by micro-thermoforming of the disposables offers unique features such as low thermal resistance for efficient thermocycling and low material consumption, which is attractive for a cost-efficient and large-scale production of disposables[4]. Microthermoforming of polymer films of typically 180 µm thickness is performed in a modified hot embossing machine, using a defined pressure and vacuum protocol. The use of this novel process has already been reported for the manufacturing of LabDisks used for sensitive subtyping of pathogenic bacteria by real-time PCR[2] and isothermal amplification of an antibiotic resistant gene of methicillin resistant *Stapphylococcus aureus* (MRSA)[3].

One objective of current R&D is to implement immunoassays and nucleic acid analysis for the detection of hazardous biological agents. The immunoassay allows the detection of ricin from blood plasma samples; the nucleic acid analysis includes a DNA/RNA extraction and an isothermal amplification for the detection of several microbial pathogens such as *Yersinia pestis* and *Bacillus anthracis*. For both assays, the complete microfluidic structures have been integrated into the foil-based disks and all the required microfluidic unit operations required to perform the assays have been verified. This includes in particular

(i) the storage of liquids and lyophilised reagents on the LabDisk and their time-controlled release,

(ii) the transfer of sample material by the use of antibody-coated microbeads and

(iii) the aliquoting of sample material for simultaneous analysis on one LabDisk.

(i) On the disposable LabDisk the required buffer solutions are stored in aluminium pouches. Applying a well-defined ultrasonic welding protocol the liquid filled pouches are equipped with a frangible seal. Due to the hydrostatic pressure of the liquids during centrifugation of the LabDisk the frangible seal bursts at a defined rotational threshold frequency of the disk and the reagents are released. Figure 2 shows a LabDisk with the microfluidic design for the immunoassay with integrated aluminium pouches for reagent pre-storage. The pouches are filled with the required washing buffers as well as with skimmed milk powder that, after rehydration, is used to block unspecific binding.

Figure 2: Disposable test carrier for immunoassay: reagents are stored in aluminium pouches. If centrifugal force is applied, the pouches burst due to the increased hydrostatic pressure of the liquid.

(ii) Both assays, the immunoassay and the nucleic acid extraction, are based on magnetic beads as mobile solid phase. Centrifugal acceleration forces mix the sample material with the magnetic microbeads, and magnetic forces allow the transfer of the microbeads between reaction chambers.

Figure 3 indicates the transfer-procedure of microbeads from the sample chamber to the detection via the washing chambers for the immunoassay. The transfer is performed automatically rotating the LabDisk over stationary magnets that are integrated into the processing device.

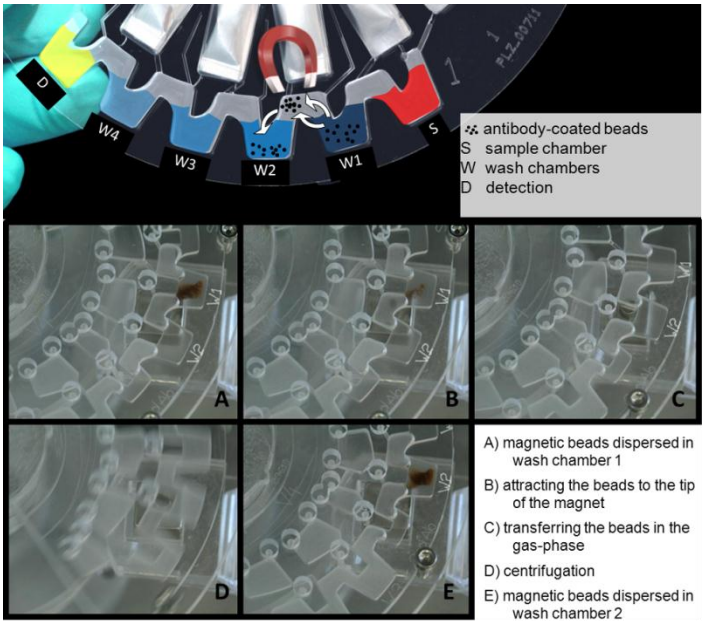

Figure 3: Transportation of magnetic microbeads in the LabDisk. The transfer of the microbeads is fully automated by rotating the LabDisk over a magnet that is integrated into the processing device.

(iii) Aliquoting of liquid sample material enables the simultaneous detection of multiple biological threats. Figure 4 shows the structure for the nucleic acid analysis including the aliquoting structure. After the extraction of DNA/RNA from blood plasma the sample is divided into multiple chambers with identical volumes for isothermal amplification of individual target sequences used for specific identification of pathogens.

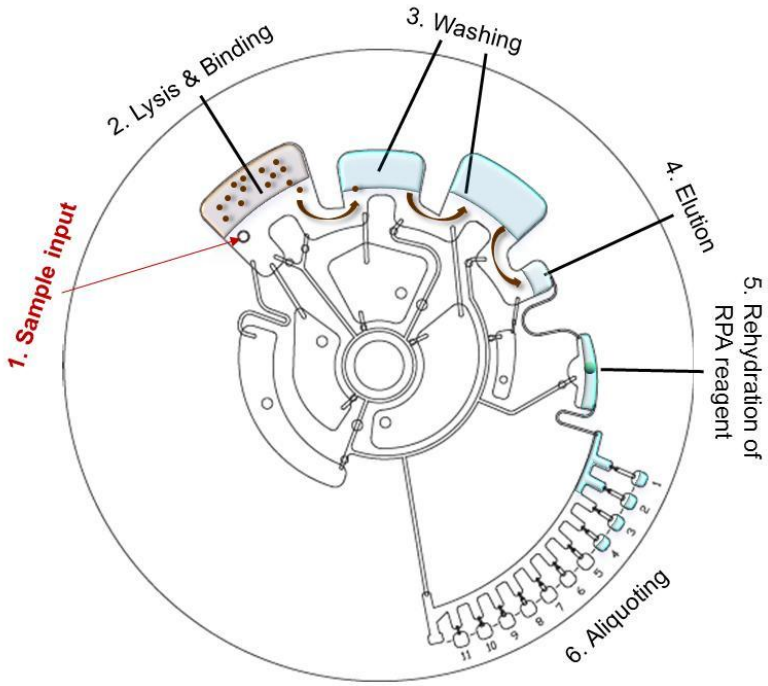

Figure 4: Process for the nucleic acid analysis including the aliquoting structure. After extraction and amplification the sample is divided into multiple chambers with identical volumes of 10 µl. Dividing the sample enables the simultaneous detection of multiple biological threats.

2. PRELIMINARY RESULTS OF INTEGRATED ASSAYS

The described operations were used to integrate two assays: An immunoassay versus the toxin Rizin and a multiplex pathogen detection assay versus (in this stage) *B. anthracis* and *F. tularensis* based on isothermal amplification and detection of DNA and RNA by Recombinase Polymerase Amplification (RPA).

2.1 Results of Immunoassay versus Rizin

The assay was a custom development sandwich ELISA by Dr. A Zgaga-Griesz and Prof. Bessler, University Medical Center Freiburg, Institute for Molecular Medicine and Cell Research. It was benchmarked in a manual laboratory protocol and compared to an automated processing on a LabDisk (Figure 5) both for Rizin in buffer and in citrated blood plasma. In the current stage, the readout is performed by absorption measurements. For that, the LabDisk had to be taken out of the processing device and readout in an external device (Wallac 1420 VICTOR2, PerkinElmer) after the process was complete. The time-to-result for the assay was approximately 45 minutes for the LabDisk assay. The assays showed similar sensitivities, although the absorption curve showed a different behaviour for the higher concentrations. The reason for that is currently being investigated. To allow readout in the processing device and completely automated testing, the assay is also converted to luminescence readout, which can be performed by the prototype processing device.

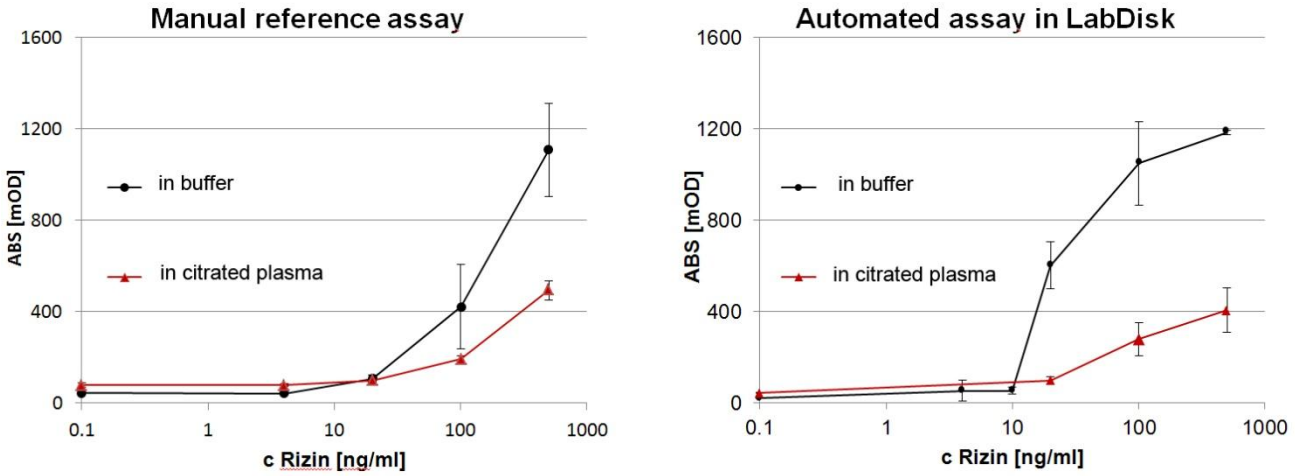

Figure 5: Results of the Rizin assay in the manual laboratory protocol (left) and processed automatically on the LabDisk (right).

2.2 Results of pathogen detection

For this assay, 10 µL of attenuated (non-infectious) *Bacillus anthracis* and *Francisella tularensis* respecitvly were spiked in blood plasma at a concentration of $3 \cdot 10^6$ genome equivalents / mL for *B. anthracis* and $3 \cdot 10^8$ genome equivalents / mL for *F. tularensis*. Detection was based on an RPA assay and fluorescent detection developed by Dr. M. Weidmann and Prof. F. Hufert, Department of Virology, University Medical Center Göttingen. This assay showed an extremely fast response (~10 minutes after sample preparation). Before the start of the assay, nucleic acid purification buffers were pipetted into the LabDisk. Then, after dispensing of the sample, nucleic acid purification, mixing with assay reagents, aliquoting, amplification and detection were all performed automatically in the LabDisk, except for the addition of the elution buffer, which has currently to be done manually during a 60 second break in the disk processing. The total assay time including sample prepration was approximately 45 minutes. Figure 6 shows the result of the assay. Clearly, both assays for *B. anthracis* (Ch_2 to Ch_5) and *F. tularensis* (Ch_6 to Ch_8) show a fast rise in fluorescence (RFU) while the chambers 1 and 9 which contained no primers and probes as negative control show no such rise. Two additional chambers 10 and 11 are currently not in use. At this time, only two targets are implemented, but the eleven chambers allow to integrate up to ten pathogens to be detected at once plus one internal control, with multiple wavelengths double or triple that amount.

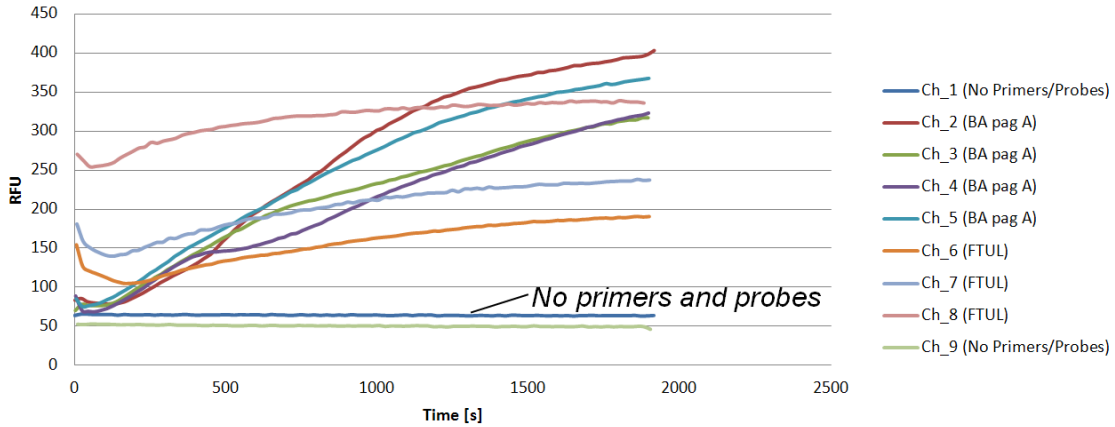

Figure 6: Results of a completely automated sample in – result out run on the LabDisk for *B. anthracis* and *F. tularensis*. The chambers 1-9 (Ch_1 to Ch_9) correspond to separated detection cavities on the LabDisk.

3. CONCLUSION AND OUTLOOK

We presented a platform for the integration and automation of immunoassays and nucleic acid assays. This allows pathogen detection and toxin quantification at the point of need without any additional laboratory equipment. At the current stage, assays for the quantification of Rizin and for the detection of *B. anthracis* and *F. tularensis* are realized. The panel will be expanded to allow the quantification of botulinum toxin and the detection of several other pathogens. This will allow to detect biohazards with a mobile device which can be operated with minimal training.

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