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RAPID FIELD TESTING OF BIOLOGICAL THREATS WITH LAB-ON-A-CHIP SYSTEMS

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Abstract
The world's increasing mobility, mass tourism but also possible terrorist activities increase the risk of a fast distribution of infections and toxins. Today's procedures for pathogen detection involve complex, stationary devices and may be too time consuming for a rapid and effective response. A robust, mobile field diagnostic system is required. A microfluidic system that includes a mobile centrifugal platform and a disposable test carrier enabling complex biochemical analysis is currently being developed within the BMBF funded project SONDE.

Project goal
In case of a pathogenic threat fast and automated field test systems are required. This need is not met by the state-of-the-art diagnostic procedures which rely on either labour-intensive and slow laboratory tests performed by skilled specialists or highly integrated but large and immobile pipetting robots.

A mobile and fully integrated diagnostic system for the detection of bacterial pathogens such as anthrax and *Y. pestis* and toxins such as ricin and botulinum toxin is about to be developed and field-tested within the project SONDE. Centrepiece of the detection system is a disposable test carrier in which reagents are pre-stored and fluidic structures are integrated to perform all required operations such as metering, mixing, valving and aliquoting. Integrated assays are performed fully automated by a centrifugal player whereby the operator requires no expert-knowledge in fluidic handling or laboratory work.

The consortium of the BMBF funded SONDE project unifies leading competences in microsystems technology and molecular diagnostics. Partners from industry and academia are:

- Robert Koch Institute, Centre for Biological Security
- QIAGEN Lake Constance GmbH
- Institut für Mikrotechnik und Informationstechnik (HSG-IMIT)
- University of Freiburg, Department of Microsystems Engineering, (IMTEK), Laboratory for Sensors
- University Medical Center Goettingen, Institute of Virology
- University Medical Center Freiburg, Institute for Molecular Medicine and Cell Research
- University of Freiburg, Zentrum für Angewandte Biowissenschaften

Prelimenary Results
Preliminary work includes the introduction of a novel foil-based fabrication method for test carriers as well as the successful implementation of analytical assays including real-time PCR and isothermal recombinase polymerase amplification (RPA) for pathogen detection.

The foil based production approach of test carriers reveals unique features like low thermal resistance for efficient thermocycling and low material consumption which is attractive for cost-efficient large-scale production of disposables (1). A novel microthermoforming process enabling production of microfluidic test carriers made out of polymer film has already been developed and reported for sensitive subtyping of pathogenic bacteria by real-time PCR (2). This rapid prototyping method allows the integration of all necessary unit operation to provide solutions for parallel analysis for the detection of B agents and toxins (Fig. 1).

The low thermal mass of foil-based test carriers fits the requirements of efficient thermocycling in real-time PCR analysis. Genotyping assays for the detection of bacterial pathogens such as methicilin-resistant *Staphylococcus aureus* (MRSA) were successfully implemented on a thermoformed test cartridge with reagents already pre-stored on disk. Performed real-time PCR tests show a limit of detection below 10 copies of DNA per reaction well (N = 24 wells in 3 independent test carriers) (2).

Fig. 1. Foil based test carrier containing thermoformed designs for real-time PCR analysis and immunoassays. Pre-stored reagents, reaction wells and read-out structures are highlighted with ink.

Regarding the requirements of mobile diagnostic systems for field testing, energy efficient solutions excel existing PCR analysis based on thermocycling protocols (3). Analyses based on the isothermal recombinase polymerase amplification (RPA) allow protocols for the detection of pathogens at constant and low temperature. We have already demonstrated a self sufficient foil-based test carrier for the fully automated analysis of nucleic acids using RPA reaction (4). The polymer foil cartridge features prestorage of all necessary liquids and dry reagents for the RPA reaction. The system was characterized with an assay for the detection of the antibiotic resistance gene *mecA* of *Stapphylococcus aureus* showing a limit of detection of < 10 copies and a time to result of < 15 minutes. Microfluidic unit operations comprise storage and release of liquid reagents, reconstitution of lyophilized reagents, aliquoting the sample into ≤ 30 independent reaction cavities, and mixing of reagents with the DNA samples (Fig. 2).

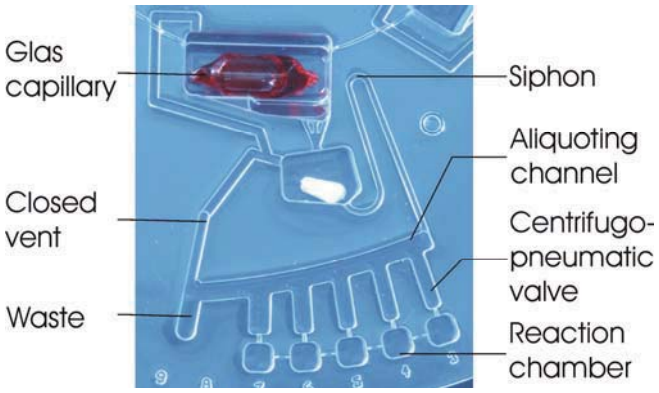

Fig. 2. Photograph of the thermoformed Lab-on-a-Chip test carrier for the detection of pathogens. The foil-based disc features a chamber with a glass capillary containing 50 µL buffer for RPA and a chamber with a lyophilisate. A capillary siphon and a centrifugo-pneumatic valve are integrated for fluid control. An aliquoting structure splits the 50 µL buffer into 5 x 10 µL.

Conclusions and Outlook

The SONDE consortium combines its competences in microbiology and microsystem technology to integrate diagnostic protocols for the automated detection of B-threats. We introduced a novel foil-based fabrication technology enabling fast prototyping of individual assays and demonstrated the suitability of microfluidic test carriers for sensitive nucleic acid analyses and immunoassays. Based on the successfully implemented biochemical analysis we are about to enhance the system integration with further unit operations such as the handling of magnetic beads in parallel to the centrifugal driven fluidic handling. Applying both, centrifugal and magnetic forces enables more complex biochemical assays such as the detection of ricin and botulinum toxin, both considered as most likely agent to be used in a biowarfare threat (5).

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References

[1] Focke, M., D. Kosse, C. Müller, H. Reinecke, R. Zengerle, and F. von Stetten (2010) Lab-on-a-Foil: microfluidics on thin and flexible films. Lab Chip 10, 1365-1386.

[2] Focke, M., F. Stumpf, B. Faltin, P. Reith, D. Bamarni, S. Wadle, C. Müller, H. Reinecke, J. Schrenzel, P. Francois, D. Mark, G. Roth, R. Zengerle, and F. von Stetten (2010) Microstructuring of polymer films for highly sensitive genotyping by real-time PCR on a centrifugal microfluidic platform. Lab Chip (in press), DOI:10. 1039/C004954A.

[3] Mark, D., S. Haeberle, G. Roth, F. von Stetten, and R. Zengerle (2010) Microfluidic Lab-on-a-Chip Platforms: Requirements, Characteristics and Applications. Chem. Soc. Rev. 39, 1153-1182.

[4] Lutz, S., P. Weber, M. Focke, B. Faltin, J. Hoffmann, C. Müller, D. Mark, G. Roth, p. Munday, N. Armes, O. Piepenburg, R. Zengerle, and F. von Stetten (2010) Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermalrecombinase polymerase amplification (RPA). Lab Chip 10, 887-893.

[5] Middlebrook, J. L. (2005) Production of vaccines against leading biowarfare toxins can utilize DNA scientific technology. Advanced Drug Delivery Reviews 57, 1415-1423.