Multiplex detection of biothreat agents using an automated electrochemical ELISA platform

Biosensors 2016

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Introduction

Modern threats of bioterrorism force the need to immediately detect and identify biothreat agents. Rapid and reliable detection of biothreat agents is of utmost importance not only to confirm that a bioterrorism event has occurred, but also to initiate appropriate organizational as well as medical countermeasures.

Methods

pBDi platform:

- Biochip based detection platform
- Integrated in a robust suitcase for use in the field by first responders (Fig. 1 A)
- Based on an automated electrochemical sandwich-ELISA (Fig. 1 B)
- Use of monoclonal capture and detection antibodies
- Capture antibodies against specific biothreat agents are immobilized on electrodes of a biochip (16 interdigitated gold electrodes)
- Multiplex detection of up to 6 biothreat agents within 20 minutes
- Inherent positive and negative control
- Very sensitive due to strongly amplified electrochemical detection system including a redox cycling process of enzyme product between interdigitated electrode structure (Fig. 1 B)
- Fully automated fluidics
- Software controlled data analysis applying a ruggedized tablet PC via USB or bluetooth connection

Fig. 2 End user test in course of GEFREASE project. pBDi operated with personal protective equipment by French and German fire fighters.

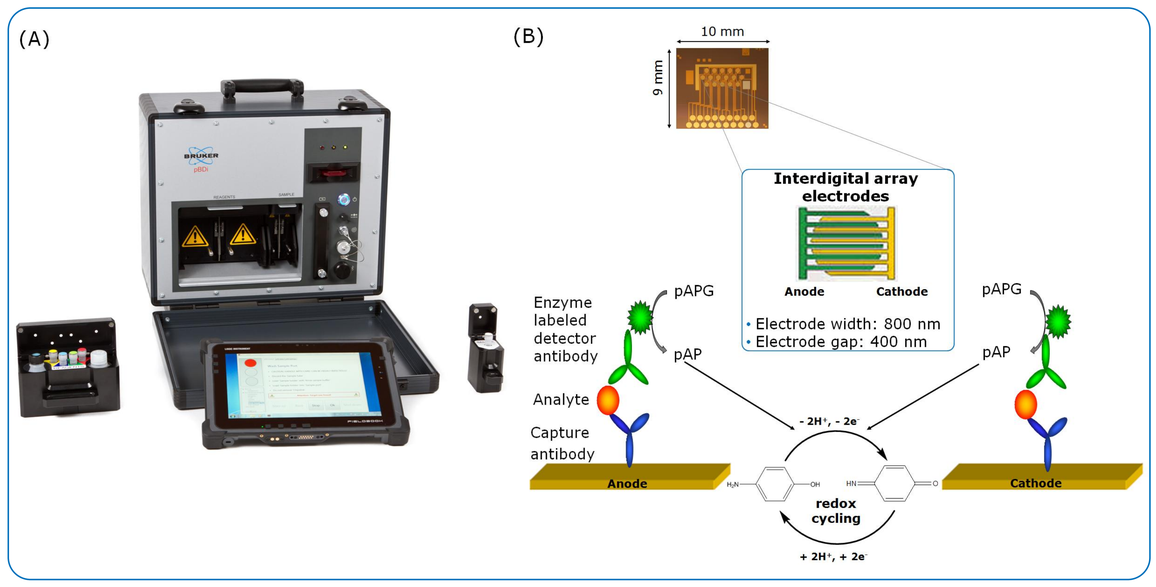

Fig. 1 The pBDi platform.

Biothreat agent	LOD[1]
B. anthracis (spores)	$1x10^5$ CFUmL^{-1}
Y. pestis	$5x10^4$ CFUmL^{-1}
F. tularensis	$1x10^3$ CFUmL^{-1}
Brucella spp.	$1x10^4$ CFUmL^{-1}
Burkholderia mallei	$1x10^4$ CFUmL^{-1}
Vaccinia virus	$9x10^4$ PFUmL^{-1}

Tab. 1 Detection limits obtained with pBDi platform. [1]LOD = Blank + 3 x SD

Results

- pBDi is usable in the field by first responders using personal protective equipment (Fig. 2).
- pBDi was evaluated by German and French fire fighters using unknown samples containing inactivated *F. tularensis* in buffer or various sample matrices, respectively. pBDi identified all unknown samples correctly.
- pBDi shows excellent limit of detections (LODs) for rapid multiplex detection of bacteria and *orthopoxvirus* (Tab. 1; Fig. 3).
- Specificity of biochip detection is demonstrated applying different amounts of bacteria or Vaccinia virus, respectively (Fig. 4).
- No detection of closely related bacteria such as *F. philomiragia*, *B. cereus, Y. pseudotuberculosis* or *Y. enterocolitica*.
- Demonstration of assay robustness detecting *F. tularensis* in sample matrices with high recovery rate (Fig. 5).
- Beside bacteria and virus detection ready-to-use kits for detection of biological toxins (e.g. ricin, botulinum toxins) available.

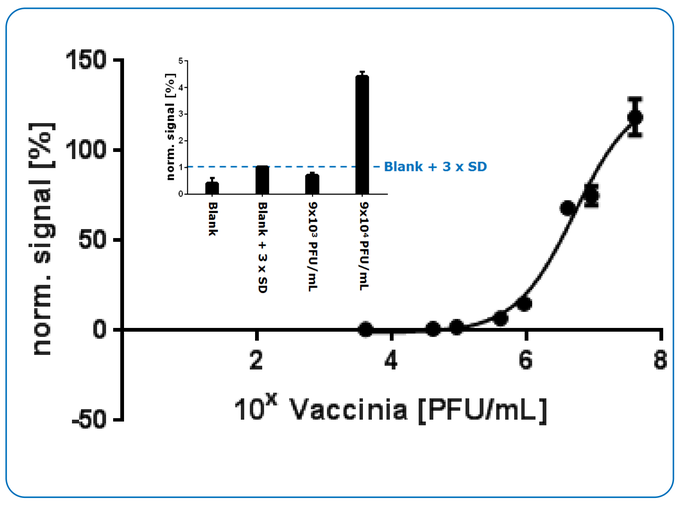

Fig. 3 Electrochemical signal as a function of Vaccinia virus concentration (n=3).

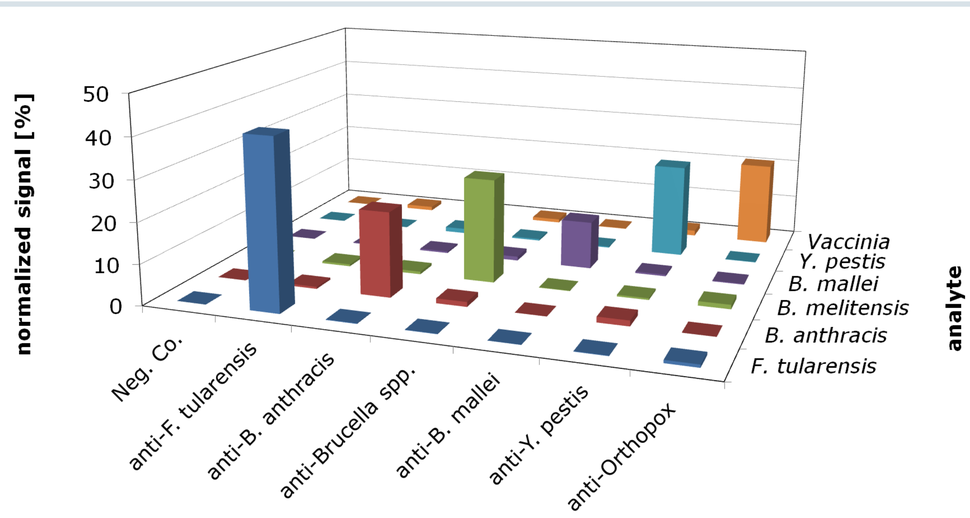

Fig. 4 Specificity of microorganism detection (n=3).

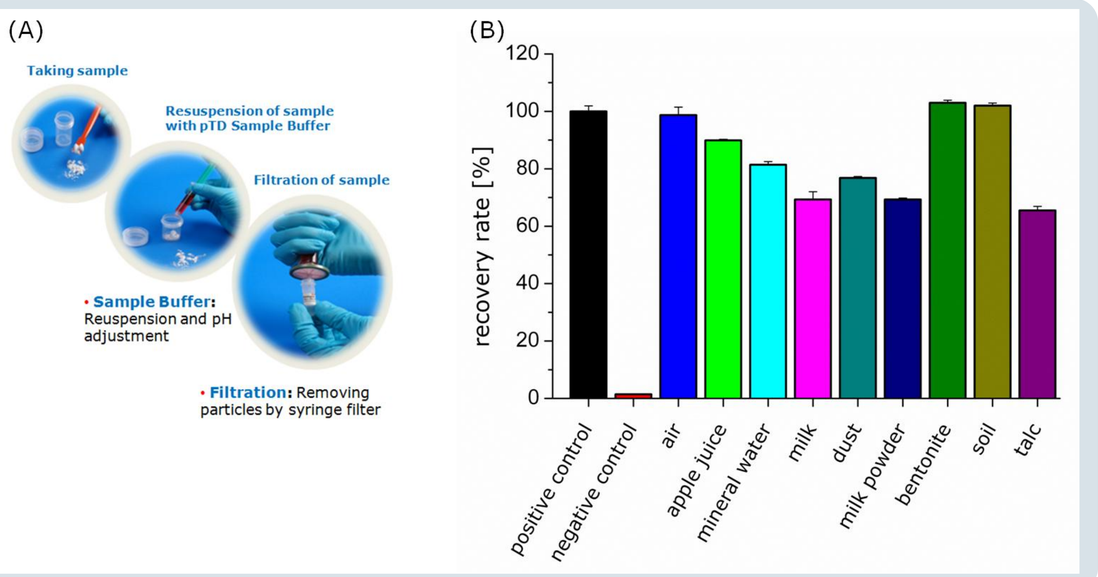

Fig. 5 (A)General sample preparation procedure. (B) Detection of $5x10^5$ CFU mL^{-1} *F. tularensis* in various sample matrices (n=3).

Summary

The results demonstrate the potential of electrochemical biochips for sensitive multiplex on-site detection of biothreat agents applying a fully automated procedure. The assays are simple to perform, show no cross-reactivity with tested agents, are rapid, and require only minimal sample preparation.

Acknowledgments

Parts of the research is funded by the Federal Ministry of Education and Research (BMBF) joint research project GEFREASE, support code 13N12222.

Conclusions

- Biothreat agents analysis in approx. 20 minutes with an automated procedure
- Parallel detection of up to six biothreat agents including positive and negative control
- Portable and vehicle integrable due to specific suitcase design
- Excellent sensitivity
- Extendable to new agents

pBDi