Field based multiplex detection of biothreat agents using electrochemical biochip technology

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Christopher Pöhlmann and Thomas Elssner
Bruker Daltonik GmbH, Permoserstrasse 15, 04318 Leipzig, Germany
C.Poehlmann@bruker.com

Introduction

Due to the potential risk of terrorist attacks using highly toxic or pathogenic biothreat agents (bacteria, viruses and toxins) there is a need for rapid, sensitive and specific detection of all kinds of biothreat agents in the field. In general, nucleic acid based detection platforms are applied for pathogen detection. However, PCR is unable to detect proteinaceous toxins and requires a clean sample demanding elaborate sample preparation. In contrast, immunoassay based technologies offer the possibility for universal detection of bacteria, viruses and toxins.

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)
- 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

Biochips: Four different biochip panels detecting each up to six biothreat agents in parallel including inherent positive and negative control electrodes are available (Tab. 1). Furthermore, biochips for site-specific immobilization of customer antibodies are available.

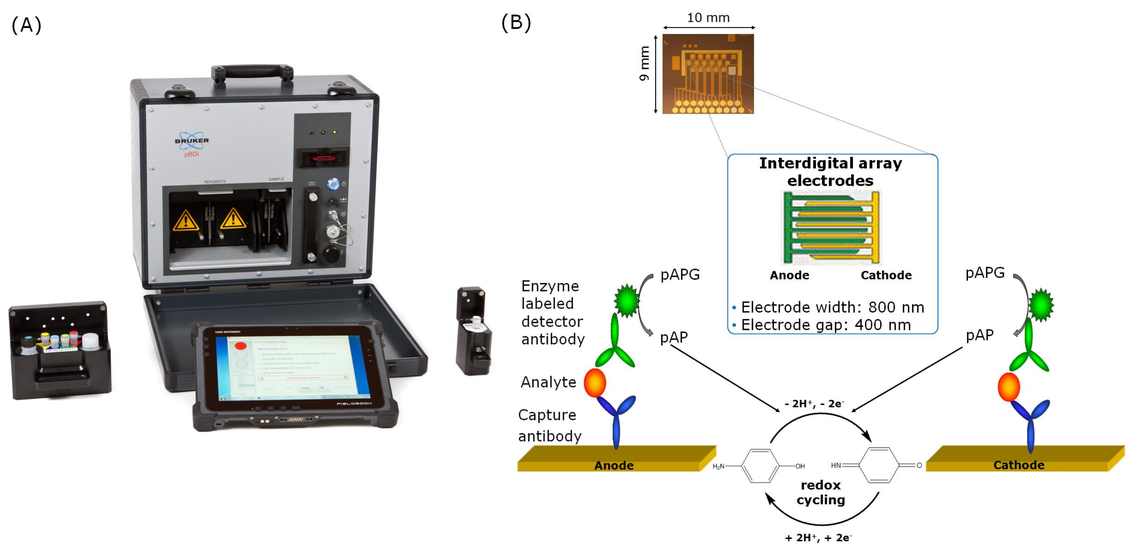

Fig. 1 The pBDi platform.

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

Biothreat agent	LOD[1]	Chip
SEA	0.25 ng mL^{-1}	Toxin chip II
SEB	0.1 ng mL^{-1}	Toxin chip I
BoNT/A	0.75 ng mL^{-1}	Toxin chip I
BoNT/B	0.5 ng mL^{-1}	Toxin chip I
BoNT/C	10.0 ng mL^{-1}	Toxin chip II
BoNT/D	2.5 ng mL^{-1}	Toxin chip II
BoNT/E	17.5 ng mL^{-1}	Toxin chip II
BoNT/F	1.0 ng mL^{-1}	Toxin chip I
Ricin	0.5 ng mL^{-1}	Toxin chip I
Abrin	1.0 ng mL^{-1}	Toxin chip II
B. anthracis	1x10^5 cfu mL^{-1}	Biothreat chip
Y. pestis	5x10^4 cfu mL^{-1}	Biothreat chip
F. tularensis	1x10^3 cfu mL^{-1}	Biothreat chip
B. melitensis	1x10^4 cfu mL^{-1}	Biothreat chip
B. mallei	1x10^4 cfu mL^{-1}	Biothreat chip
Vaccinia	9x10^4 pfu mL^{-1}	Biothreat chip
V. cholerae	1x10^3 cfu mL^{-1}	Food chip
S. dysenteriae	1x10^6 cfu mL^{-1}	Food chip
C. jejuni	7x10^4 cfu mL^{-1}	Food chip
S. typhimurium	1x10^6 cfu mL^{-1}	Food chip
E. coli O157:H7	1x10^4 cfu mL^{-1}	Food chip
L. monocytogenes	5x10^4 cfu mL^{-1}	Food chip

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 20 unknown samples containing varying amounts of toxin or bacteria in buffer or various sample matrices, respectively. pBDi identified all 20 unknown samples correctly.
- pBDi shows excellent limit of detections (LODs) for rapid multiplex detection of bacteria, *orthopoxvirus* and toxins (Tab. 1).
- Specificity of biochip detection is demonstrated applying different amounts of bacteria or *Vaccinia* virus, respectively (Fig. 3).
- Demonstration of assay robustness detecting ricin or *F. tularensis*, respectively, in sample matrices with high recovery rate (Fig. 4).

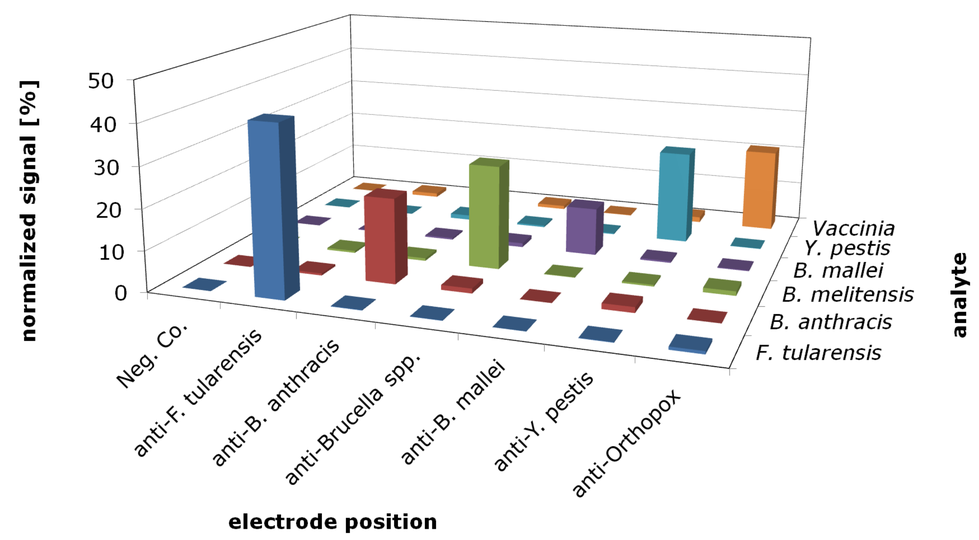

Fig. 3 Specificity of microorganism detection.

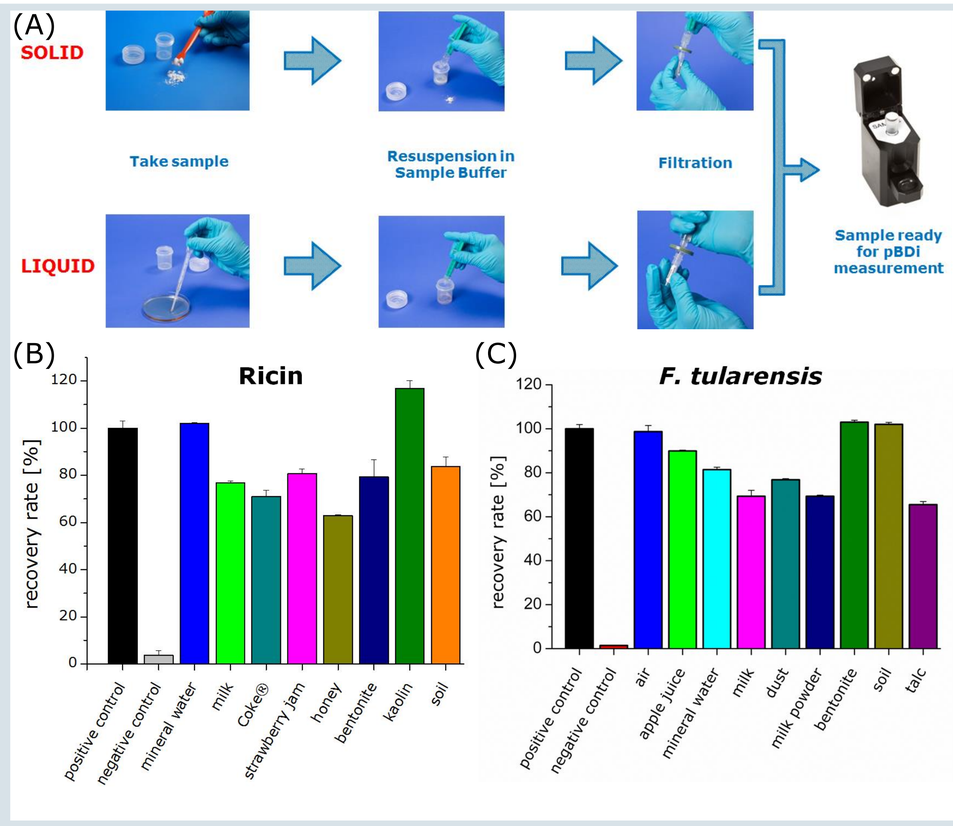

Fig. 4 (A) Sample preparation procedure.
(B) Detection of 10 ng mL^{-1} ricin in different sample matrices.
(C) Detection of 5x10^5 cfu mL^{-1} *F. tularensis* in various sample matrices.

Acknowledgments

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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