Application Note
#CBRNE - 1849594

Simultaneous detection of six biothreat agents using the portable BioDetector pBDi

The portable BioDetector integrated (pBDi) allows the rapid, specific and sensitive identification of pathogenic organisms, which are relevant as biological warfare agents. Application of the ready-to-use pBDi Biothreat Test Kit 1 allows the parallel detection of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Brucella* spp., *Burkholderia mallei* and smallpox virus within 20 min through an automated process. Highly robust electrochemical biochip technology guarantees reliable identification of biothreat agents in solid, liquid and aerosol samples, requiring only minimal sample preparation.

Introduction

The danger of emergencies involving biothreat agents is ever present and can occur because of a natural catastrophe, a localized outbreak (epidemic) or a worldwide outbreak (pandemic) of an infectious agent, warfare, or acts of terrorism. Rapid and reliable identification of biothreat agents is of utmost importance not only to confirm that a bioterrorism event has occurred, but moreover to initiate appropriate organizational as well as medical countermeasures. The pBDi is a portable detection platform for automatic, rapid and sensitive on-site identification of up to six biothreat agents in parallel, including inherent positive and negative controls.

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Biothreat agents detection	pBDi
Biochip	pBDi Control
Immunoassay	

The pBDi is based on the same electrochemical ELISA (enzyme-linked immunosorbent assay) biochip technology as the Bruker pTD (portable Toxin Detector) [1] bench top device, enhanced by new features such as integration in a case, battery mode as well as bluetooth connection to a ruggedized tablet PC. A wider selection of biochips for detection assays for detection of bacteria, viruses and toxins completes the enhancement.

The application of this technology was recently demonstrated for detection of toxins such as ricin or staphylococcal enterotoxin B (SEB) in unknown samples [2; 3]. In this application note, we introduce the pBDi for the simultaneous detection of pathogens associated with biological warfare agents.

Experimental

All cultivation and inactivation steps were performed in a level 3 biosafety laboratory. Cultivation of different bacteria was performed according to standard microbiological techniques. *Bacillus anthracis* spores were inactivated according to Kuehn *et al.* (2009) [4]. *Yersinia pestis* grown at 28°C or 37°C, respectively, were inactivated either by heat treatment or by inactivation with formalin according to Tomaso *et al.* (2007) [5]. All other bacteria were inactivated using heat treatment. Inactivation was confirmed by plating of inactivated bacteria suspension on agar plates and incubation at 37°C for at least 48 h. No bacterial colonies were observed after the dedicated inactivation procedures were completed.

Vaccinia virus suspension inactivated using ß-propiolactone was provided by the Commissariat à l'énergie atomique et aux énergies alternatives (Dr. Laurent Bellanger, Laboratoire Innovations technologiques pour la Détection et le Diagnostic, Bagnols-sur-CézeCèze, France).

Optical density at 600 nm was used for the determination of colony forming units (CFU) according to a standardized growth curve. Bacteria counts were verified by plating decimal dilution series in 0.9% NaCl on agar plates, incubated for 24 h at 37°C or 28°C respectively.

Standardized soil samples were obtained from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer (Germany), whereas dust was provided by the Robert Koch Institute (Dr. Brigitte Dorner, Centre for Biological Threats and Special Pathogens, Biological Toxins, Germany). Foodstuffs were purchased from local grocery stores.

In order to conduct biothreat agents detection; the pBDi with the pBDi Control software and the pBDi Biothreat Test Kit 1 (Bruker Daltonics, Germany) containing all reagents (enzyme, substrate, neutralizer, detection antibody mix and decontamination solution) and buffers was used.

For preparation of artificially contaminated samples inactivated *F. tularensis* was added to a 10% (w/v) solid sample matrix or to a 50% (v/v) liquid sample matrix in sample buffer. Suspension was incubated for at least 30 min at 25°C in an end-over-end shaker (HULAmixer® sample mixer, Thermo Fisher Scientific, Waltham, MA, USA) and filtered through a syringe filter before application to the pBDi. All buffers and consumables necessary for sample preparation are included in the pBDi Sample Preparation Kit.

Results

Pathogen detection with the pBDi

Besides the sensitive detection of toxins already demonstrated previously [2; 3] the pBDi can also be used for the determination of pathogens such as bacteria and viruses when deployed in the field. A biochip carrying 16 electrode positions was used for immobilization of monoclonal antibodies raised against *B. anthracis, Y. pestis,*

Table 1: Specificity of biothreat agent detection using pBDi and pBDi Biothreat Test Kit 1.

Highly specific biothreat agents detection

	Electrode position					
Analyte	**F. tularensis**	**B. anthracis**	**Brucella spp.**	**B. mallei**	**Y. pestis**	**Smallpox**
F. tularensis subsp. *tularensis*	+	Ø	Ø	Ø	Ø	Ø
F. tularensis subsp. *holarctica*	+	Ø	Ø	Ø	Ø	Ø
B. anthracis spores	Ø	+	Ø	Ø	Ø	Ø
B. melitensis	Ø	Ø	+	Ø	Ø	Ø
B. abortus	Ø	Ø	+	Ø	Ø	Ø
B. suis	Ø	Ø	+	Ø	Ø	Ø
B. mallei	Ø	Ø	Ø	+	Ø	Ø
B. pseudomallei	Ø	Ø	Ø	+	Ø	Ø
Y. pestis (cultivated at 28°C)	Ø	Ø	Ø	Ø	+	Ø
Y. pestis (cultivated at 37°C)	Ø	Ø	Ø	Ø	+	Ø
Vaccinia virus WR	Ø	Ø	Ø	Ø	Ø	+

+ positive result, i.e. normalized signal [%] above threshold value of 3%; Ø negative result, i.e. normalized signal [%] below threshold value of 3%.

F. tularensis, Brucella spp., B. mallei and orthopox viruses. Capture antibodies specific for an agent were immobilized on two electrode positions each. These redundant sensor positions and two additional internal positive and negative controls each greatly reduce the false alarm rate.

Detection of bound biothreat agents is realized by the application of a detector-antibody-enzyme conjugate and measurement of the electrical current of an enzymatic redox reaction on the interdigitated electrodes. The detection event is strongly amplified in this system and allows very sensitive biothreat agent identification in approx. 20 minutes. Initially the high turnover of enzymatic reaction contributes to the signal amplification and secondly, a redox cycling procedure built into the experimental procedure, provides a second signal amplification [6]. Table 1 shows bacterial and viral species detectable by means of the pBDi Biothreat Test Kit 1.

Specificity of biothreat agent detection

Specificity of simultaneous detection of bioterrorism relevant pathogens is summarized in Table 1. Specificity was assessed individually with high concentrations (≥ $5.0x10^6$ CFU mL^{-1} or $5.0x10^6$ PFU mL^{-1}) of the relevant species of biothreat agents. Each target pathogen produced significant signals only on electrodes immobilized with the respective specific capture antibodies. No significant cross-reactivity of antibodies with analyzed target substances was observed confirming the high specificity of the assay.

Sensitivity of biothreat agent detection

The performance of the pBDi Biothreat Test Kit 1 was evaluated applying serial dilutions of pure cultures of target

Sensitivity of biothreat agent detection

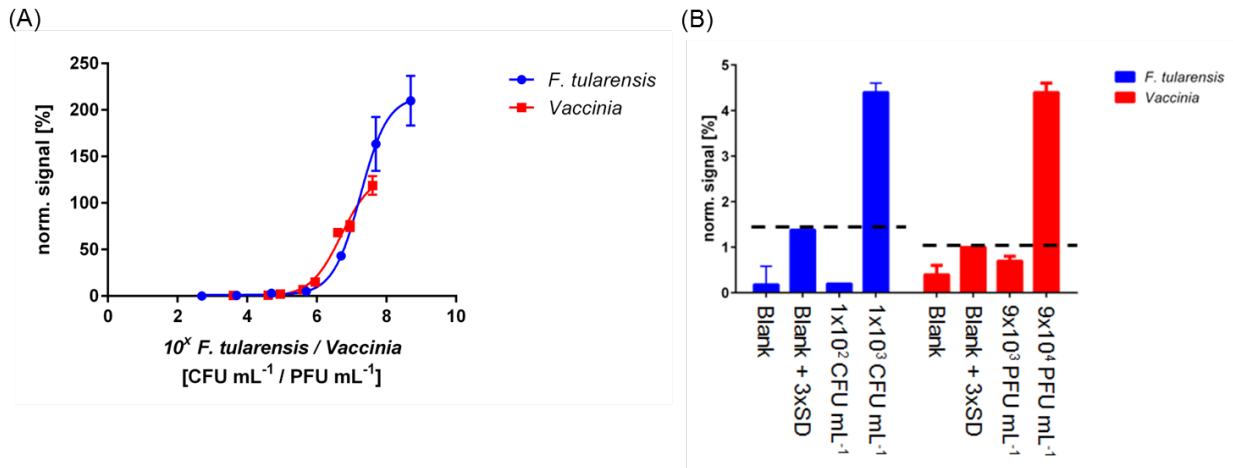

Figure 1: Sensitivity of detection of *F. tularensis* or *Vaccinia* virus, respectively, using pBDi in combination with pBDi Biothreat Test Kit 1. (A) Different concentrations of inactivated *F. tularensis* subsp. *tularensis* SCHU S4 (FSC237) (blue) or inactivated *Vaccinia* virus (red) in sample buffer were applied for electrochemical measurement. (B) Determination of the limit of detection for *F. tularensis* subsp. *tularensis* SCHU S4 (FSC237) (blue bars) or inactivated *Vaccinia* virus (red bars). Dashed line indicates the value for blank measurement plus three times the standard deviation of blank measurement.

Straightforward sample preparation procedure

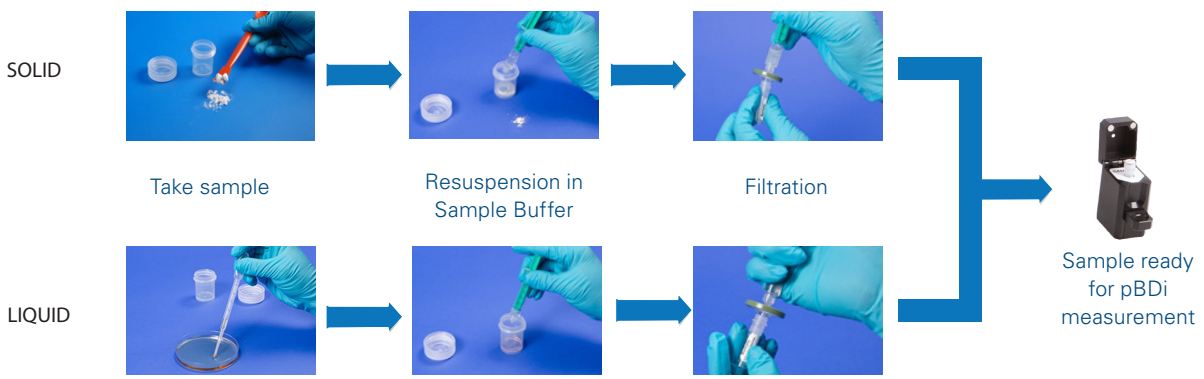

Figure 2: Workflow for sample preparation of liquid and solid samples for subsequent measurement with the pBDi platform.

pathogens. The minimum concentration detected above a threshold value (mean blank plus three times standard deviation of blank value) was defined as the limit of detection (LOD). The results show that the LOD of the assays are in the range of 10^3 to 10^5 CFU mL^{-1} depending on target pathogens. Figure 1 displays a sigmoidal shaped standard curve for bacterial agent *F. tularensis* or *Vaccinia* virus, respectively, as well as electrochemical signals at the LOD.

Application of pBDi Biothreat Test Kit 1 using environmental, food and powder sample matrices

Efficient sample preparation, i.e. isolation of pathogens from complex samples, is crucial for the subsequent precise detection of analytes. The extent of sample preparation depends upon the analytical method applied. An advantage of immunoassay based detection methods, is that less sample preparation is often needed prior to analysis compared to nucleic acid amplification based techniques. This is due to the fact that surface antigens were detected and no sophisticated extraction and purification steps have to be performed. A straightforward workflow for liquid and solid samples, using the dedicated pBDi Sample Preparation Kit; allows highly robust and interferent free detection of biothreat agents using the pBDi system (Figure 2).

Complete sample preparation can be accomplished within 2 to 5 min in the field by first responders without any need for additional equipment.
Biological threat agent detection using the pBDi system has been successfully demonstrated in environmental samples (e.g. aerosol samples, soil, dust), food samples (e.g. milk, apple juice, mineral water) and powder samples (e.g. talc, milk powder, bentonite) (Figure 3) showing excellent recovery rates even in complex sample matrices like soil or milk.
Furthermore, during the GEFREASE project (BMBF/ANR funded project) fire brigades and police from Germany and France tested the pBDi system successfully wearing personal protective equipment. 10 unknown samples containing varying amounts of bacteria (heat inactivated *F. tularensis*) in buffer or various sample matrices were correctly identified.

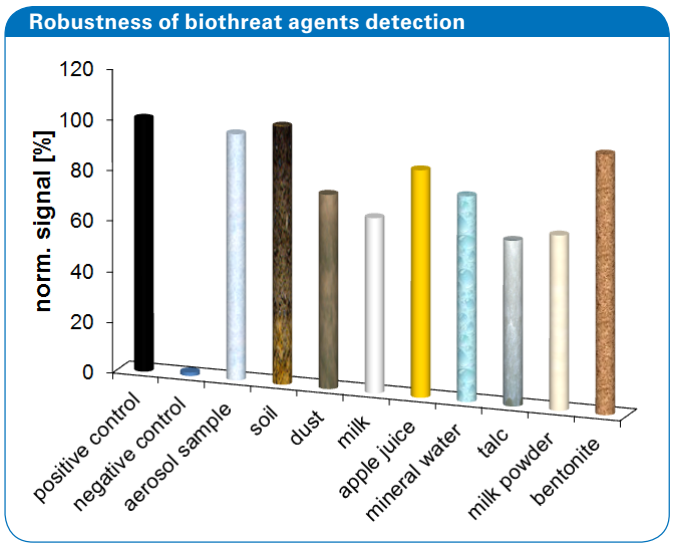

Figure 3: Application of pBDi Biothreat Test Kit 1 for on-site pathogen detection in various sample matrices. Detection of $5x10^5$ CFU mL^{-1} *F. tularensis* in various sample matrices.

Conclusion

The Bruker pBDi is a portable detection platform for rapid and sensitive on-site identification of biothreat agents. Developed for use by non-scientific personnel, the pBDi is easily operated, even when working in protective equipment under extreme conditions. Fully portable and operating from internal batteries, pBDi can be used in the hot zone. Equally, pBDi can be integrated with various mobile platform solutions and can be powered from an external or on-board supply.

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