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Abstract

Integration of a Bead-Based Immunoassay on a Commercial PCR-Performing POC Device †

Benita Johannsen [1], Desirée Baumgartner [1], Michal Karpíšek [2,3], David Stejskal [4,5], Nils Paust [1,6], Roland Zengerle [1,6] and Konstantinos Mitsakakis [1,6,*]

[1] Hahn-Schickard, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
[2] BioVendor-Laboratorní Medicína a.s., Research & Diagnostic Products Division, Karasek 1767/1, Reckovice, 62100 Brno, Czech Republic
[3] Faculty of Pharmacy, Masaryk University, Palackeho trida 1946/1, 61242 Brno, Czech Republic
[4] Department of Biomedical Sciences, Faculty of Medicine, University of Ostrava, Syllabova 19, 70300 Ostrava, Czech Republic
[5] Institute of Laboratory Diagnostics, University Hospital Ostrava, 17. listopadu 1790/5, 70800 Ostrava, Czech Republic
[6] Laboratory for MEMS Applications, IMTEK—Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
* Correspondence: konstantinos.mitsakakis@hahn-schickard.de; Tel.: +49-761-203-73252
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Abstract: Point-of-care (POC) infectious disease diagnostic devices typically detect either only pathogens or only protein biomarkers, while they provide a low throughput of samples (one to two) tested per run. In this work, we demonstrate the adaptation of a bead-based competitive immunoassay method to be compatible with a commercial POC PCR-performing device without modifications to its hardware. In addition, the assay integration in two half disk-shaped cartridges with seven identical structures on each provides a considerable throughput of 14 samples per 20 min run.

Keywords: immunoassay; bound-free phase; micro/nanoparticles; point of care; microfluidics

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1. Introduction

In the field of infectious diseases, biomarker detection in automated systems is essential for co-assessment with pathogen detection towards an accurate, evidence-based decision for antibiotic prescription and the transfer of diagnostics from the central laboratory to the point of care (POC) [1]. Along this line, we demonstrate the integration of a bound-free phase detection immunoassay (BFPD-IA) [2] (Figure 1) in a half-disk-shaped cartridge in a way that a throughput of 14 samples per run is achieved, and a commercial POC PCR-performing instrument can process this ImmunoDisk. An indicative clinical scenario of detecting C-reactive protein (CRP) in the case of respiratory tract infections (RTIs) was examined using the BFPD-IA on a microtiter plate reader (MTP) and a commercial PCR device.

2. Materials and Methods

Details on the BFPD-IA operating principle and protocols for the CRP assay, and the coupling of magnetic and fluorescent beads (MB; FB) with antibodies and competitive antigens, respectively, are available in earlier work [2]. Here, we adapted the dry storage of MB in the disk at 43 °C for 0.5 h using TRIS with 25% (*w*/*v*) sucrose, 25% (*w*/*v*) PEG1000 and 0.1% (*w*/*v*) CHAPS. The ImmunoDisks were fabricated via the microthermoforming of polycarbonate foils, carried out as described before [3], and were tested on the commercial Rhonda POC PCR device (Spindiag GmbH, Freiburg, Germany).

3. Results and Discussion

The core of BFPD-IA microfluidic integration is a 'multipurpose' chamber, which serves the pre-storage of MB, the assay incubation based on a combination of shake mode and magnet mixing, the separation of MB, and the detection of unbound FB. A half disk-shaped cartridge consisted of seven such chambers, each connected to its own inlet for inserting the sample, assay buffer, and FB (Figure 1). Two half-disks were run simultaneously on the Rhonda device, providing results in 20 min. The inter-chamber reproducibility was tested by running, in all chambers, an assay between the MB-coupled antibodies and the FB-coupled competitive antigens, resulting in a coefficient of variation (CV) of 5.4 %. Standard curves were acquired, keeping the sample and the MB volumes constant at 1.5 and 5.0 µL, respectively, while using different FB volumes, with 3.0 µL performing best due to it having the highest signal ratio between max and low concentration (ratio 4.2, versus 3.4 for 4.0 µL FB, Figure 2). Based on a non-linear curve fit, the limit of detection was calculated at 2.3 mg/L, clearly below 20 mg/L, which is the low cutoff value of RTI-relevant clinical guidelines for CRP [1]. Another cutoff is 40 mg/L, which is for exacerbations of chronic obstructive pulmonary disease (COPD) at the general practice (GP) [4]. These two concentrations create three 'zones'. We measured 27 clinical serum samples using BFPD-IA on benchtop MTP and the disk on Rhonda, and classified them in these concentration zones, A, B, and C (Figure 2). The two methods agreed in 22/27 cases. These promising preliminary results encourage us to proceed towards increasing the integration degree for sample-to-answer analysis, while retaining the throughput degree, and implementing the system in additional clinical scenarios, e.g., sepsis, and in broader, comprehensive clinical studies. Eventually, performing an immunoassay on the same instrument as the one that PCR is performed, can potentially save costs and improve logistics in diagnostics.

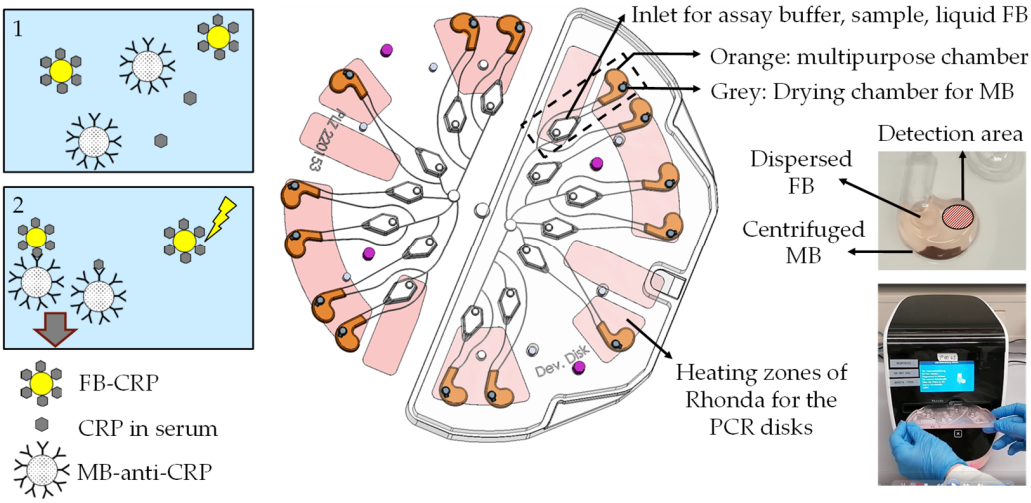
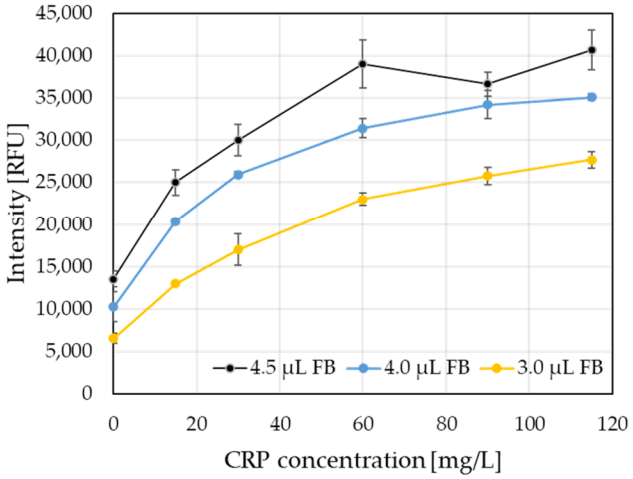


Figure 1. Left: Schematic of the BFPD-IA. The grey arrow and yellow symbol in step 2 indicate the separation of the MB (e.g., via centrifugation) and the fluorescence detection, respectively. Middle: Design of two half-disks with 14 multipurpose chambers. The dashed box indicates the repeated structure. Bottom right: a Rhonda device.



A: ≤20 B: 20-40 C: >40		CRP on MTP		
		A	B	C
CRP on disk	A	4		
	B	2	6	
	C		3	12

Figure 2. Left: Standard curves with varying FB volumes. Right: The table indicates the number of samples that were classified in each concentration zone A, B, and C (values in mg/L), based on the MTP and the disk on Rhonda.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by The Ethics Committee of Faculty Hospital Ostrava (Protocol Code Number: TACR 01/2020-12-2023 from 19 March 2020; reference number 297/2020) from 19 March 2020.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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