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Fast multiplex analysis of antibodies in complex sample matrix using the microfluidic Evalution™ platform

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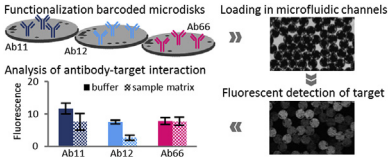
HIGHLIGHTS

- The Evalution™ platform was evaluated for high-throughput immunoassay development.
- Fast and multiplex analysis of antibodies' performance was shown using Evalution™.
- The obtained results were in accordance with ELISA and SPR measurements.
- Influenza A nucleoprotein was detected on Evalution™ in nasopharyngeal swabs.
- The detection limits for nucleoprotein were comparable to standard ELISA

GRAPHICAL ABSTRACT



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ABSTRACT

Biosensor development requires comprehensive research for establishing the optimal bioassay conditions that determine the final kinetics, sensitivity and specificity. Different systems have been developed to study bioreceptor-target interactions but they often have drawbacks, such as long hands-on time, low throughput, high sample consumption and high cost. In this work, the potential of the novel microfluidic Evalution™ platform has been evaluated for developing sandwich-based assays in a fast and high-throughput fashion. An immunoassay for the detection of influenza A nucleoprotein was used as a model system. Exploiting the platform's unique features, various typical bioassay parameters (e.g. aspecific binding between assay components, different reagent concentrations and incubation times) were tested for three capture antibodies in a simple and fast manner (2 runs of 80 min). The selected conditions, giving the highest signal-to-noise ratio, were directly employed on the same platform to detect nucleoprotein in buffer and nasopharyngeal swabs. Two antibodies with a higher dissociation constant (Ab11 and Ab12) required longer incubation times (60 min) for sensitive detection (limit of detection (LOD) of 0.48 and 0.26 ng mL^{-1}, respectively) compared to an antibody with lower dissociation constant (LOD of 0.04 ng mL^{-1} for Ab66 within 30 min). Moreover, one antibody (Ab12) showed limited capacity to capture nucleoprotein directly in sample matrix. The obtained results were in accordance with previous studies performed on an ELISA and SPR platform with the same antibodies. This positions the Evalution™ platform as a reliable platform for fast and multiplex analysis of antibodies' performance both in buffer and complex sample matrices.

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1. Introduction

Bioassay development is one of the most crucial aspects for obtaining a functional biosensor and requires a methodological approach to select the ultimate assay components and conditions. Specifically, the bioreceptors have a major influence on the final assay performance since they determine not only the kinetics of the binding reaction but also the sensitivity and specificity of the interaction. Moreover, their performance in complex sample matrices defines the applicability of the developed bioassay in real samples. Ideally, receptors should bind to the target with fast kinetics, while discriminating it from other abundantly present sample components. To achieve this, multiple bioreceptors need to be tested for their interaction with the target under different conditions in order to select the most prominent candidate for the assay.

Different techniques are available for the analysis of bioreceptor-target interactions, with enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) [1,2] being most commonly used. In a typical ELISA, the target is sandwiched between two antibodies and an enzyme is used to generate signal [3]. Although simple in its format while providing high throughput, standard ELISAs require significant handling time, have long time-to-result (because the different incubation times are largely dependent on diffusion), have high sample consumption and provide only endpoint measurements [1]. SPR is an alternative label-free technique used to monitor interactions of molecules (e.g. protein-protein, DNA-protein). Although it allows real time analysis in a short assay time, standard commercial SPR devices (e.g. Biacore) still have a low throughput, due to the limited number of flow channels, and low multiplexing capacity. Moreover, the measurements usually have a large reagent consumption, thereby increasing the assay cost and requiring a larger sample volume [2].

During the last couple of years, several methods have been developed to overcome the above mentioned disadvantages of the standard ELISA and SPR platforms. Thus, the size of bulky prism-based SPR instruments has been reduced, resulting in less sample consumption and decreased equipment and analysis cost. However, this comes at a price since the sensitivity of these systems is generally lower compared to the standard SPR systems [4–7]. Other techniques providing bioreceptor-target interaction analysis, such as dynamic force spectroscopy and microscale thermophoresis, consume limited amount of sample and allow single molecule analysis but have a very low throughput [8,9]. Recently, significant effort has been invested in developing platforms with high multiplexing capacity since it has a major influence on the total assay time and cost of the analysis system [3]. One of the most commonly used is the xMAP technology of Luminex. Here, magnetic beads are color coded with specific fluorophores and coated with antibodies to capture the target, which leads to signal generation that can be detected using flow cytometry or CCD imaging. Multiple sandwich immunoassay kits using the xMAP technology are commercially available [10]. Another possibility is printing multiple antibodies on specific spots on an antibody array and analyzing the interaction of each target with their corresponding spot (e.g. RayBiotech, Quansys Biosciences) [11,12]. Although these techniques allow high multiplex analysis, they still require significant hands-on time. To reduce the handling time and sample consumption, some microfluidic platforms have been successfully integrated with standard ELISA but have only limited multiplexing [13,14].

Recently, a new platform called Evalution™ (MyCartis NV, Belgium) has been launched that uses barcoded microparticles (μPs) in a fully automated microfluidic environment. While this platform combines fast assay time with a high level of multiplexing (up to 150-plex in one channel and the possibility to run up to 16 channels simultaneously), it also reduces sample consumption because the flow in the channels is generated by a pressure difference, thus avoiding the use of tubing. The instrument provides dynamic control over assay conditions by allowing real-time data processing and display. Because of all these features, Evalution™ has the potential to be used not only for bioassay development but also for target detection. Although the standard ELISA and SPR systems can also be used as diagnostic tools [15–18], the ongoing trends in the diagnostic field towards point-of-care tests and personalized medicine reduce their usability in those fields [19]. The first example showing the capacity of the Evalution™ platform as a diagnostic tool was the sensitive detection of cytokines in a 9-plex format [20].

In this work, the Evalution™ platform has been evaluated for its potential for developing sandwich-based assays in a fast and high-throughput fashion both in buffer as well as in complex sample matrix. A sandwich immunoassay for the detection of influenza A nucleoprotein is used as a model system. Previously, our group has used the same model on ELISA and SPR platforms [21] and hence these results can be used to benchmark the results obtained on Evalution™. Using the possibility to test multiple parameters in a single run, several analyses were performed to: (i) monitor the aspecific interaction between assay components and increase the signal-to-noise ratio, which is crucial in bioassay optimization; (ii) test the effect of varying incubation times of the nucleoprotein and (iii) test the assay performance using a complex sample matrix, namely nasopharyngeal swabs.

2. Material and methods

2.1. Reagents

All reagents were purchased from Sigma-Aldrich (Belgium) unless stated otherwise. Based on previously published work [21], three commercially available monoclonal mouse antibodies (ab110661, ab128193 and ab66191) were selected for analyzing their binding affinity towards recombinant influenza A nucleoprotein, in this manuscript referred to as Ab11, Ab12 and Ab66, respectively. All monoclonal antibodies were raised against nucleoprotein isolated from real virus and purchased from Abcam plc. (United Kingdom). Polyclonal rabbit antibody against nucleoprotein (11675-RP01, referred to as secondary antibody) was purchased from SinoBiological Inc. (China). Biotinylated goat-anti-rabbit antibody (A16114, referred to as detection antibody) was obtained from Thermo Fisher Scientific Inc. (United States). Streptavidin labeled with phycoerythrin (SAPE-001, referred to as SA-PE) and buffer diluent (PECD-100) were purchased from Moss Inc. (United States). LowCross buffer (100 500) was obtained from Candor Bioscience GmbH (Germany). Custom made recombinant influenza A nucleoprotein (IMR-274) was purchased from Imgenex (United States).

2.2. Evalution™ instrument

The Evalution™ platform relies on three major components, being barcoded μPs, microfluidic assay cartridges and an instrument for the integration of all assay steps [20]. The μPs are silicon discs with a diameter of 40 μm and a thickness of 10 μm. They are barcoded with 10 binary coding bits on their periphery, enabling 1024 (2^{10}) different codes. The central area is functionalized to allow coupling of biomolecules and is dedicated to measure fluorescence intensity. By the immobilization of different capture molecules onto differently encoded μPs, mixed μP populations can be prepared, creating a multiplex environment. These μPs are loaded into a microfluidic cartridge consisting of 16 microfluidic

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