AUTOMATION OF HETEROGENEOUS PROSTATE CANCER IMMUNOASSAY BASED ON MULTI-VALVE TRIGGERING USING PURELY ROTATIONAL FLOW CONTROL

R. Mishra[1], J. Zapatero-Rodríguez[2], S. Sharma[2], D. Kelly[2], D. McAuley[1], R. O'Kennedy[2] and J. Ducrée[1,2]

[1]School of Physical Sciences, Dublin City University, Ireland and
[2]Biomedical Diagnostics Institute, Dublin City University, Glasnevin, Ireland

ABSTRACT

A multi-marker approach is increasingly considered in diagnosis of prostate cancer to minimize the number of unnecessary biopsies [1]. Centrifugo-pneumatic flow control is often the method of choice for multiplexing such assays [2]. However, the number of assays that can be parallelized on such a dissolvable-film (DF) based system is limited by the substantial spread of the burst frequencies [2] owing to manufacturing tolerances. In this work we substantially enhance the definition of actuation frequencies by 75% using a novel design to enable the automation of multi-step / multi-reagent Lab-on-a-Disc (LoaD) platform. We demonstrate a multi-marker prostate cancer immunoassay panel.

KEYWORDS: Immunoassays, Centrifugal microfluidics, dissolvable film valving, prostate cancer

INTRODUCTION

Centrifugal microfluidic systems for decentralized testing at the point-of-care employ rotationally induced sample preparation and flow control which can be implemented on a rugged and cost-efficient instrument [1]. We recently introduced a new, rotationally controlled flow technology based on disc-incorporated DF membranes that offers decisive benefits [2]. However, the substantial spread of the burst frequencies which is linked to unavoidable manufacturing tolerances remains a limiting factor [2]. In this work we substantially enhance the definition of actuation frequencies by 75% (from an average of ±7.5 Hz down to ±1.8 Hz) using a novel design to enable the comprehensive automation of multi-step / multi-reagent immunoassay panels on the LoaD platform. As a pilot application, we implement the sample handling required for the detection of two prostate cancer markers (fPSA and Her2 ECD) with two different Enzyme-Linked Immuno-Sorbent Assays (ELISAs).

EXPERIMENTAL

The entire sample preparation of three parallel assays from whole blood is solely orchestrated by changes in the spin speed of the LoaD cartridge featuring specifically designed DF valves (Fig. 1). Figure 2 shows the novel design for the DF valving which employs: a by-pass chamber ensuring a controlled rise of liquid before reaching the DF valve; a trigger lip placed at the end of this bypass chamber that directly opens to the recess of the DF tab; a separately connected pneumatic chamber the volume of which sets the burst frequency of the valve (in accordance with the Boyles law); and a peripherally placed DF valve that allows immediate dissolution of the DF upon the entry of the first liquid.

RESULTS AND DISCUSSION

The new valve design (Fig. 2) considerably improves the reliability, thus permitting a higher integration density for increasing the number of bioassays that can be parallelized on a single disc cartridge as in Figure 1. Figure 3 shows the results for fPSA and Her2 detection from buffer on the LoaD for a run time of 53 minutes.

CONCLUSION

While the emphasis of this work is on the fluidic automation of the multiplexed assay, we are already able to demonstrate the detection of Her2 and fPSA within and near the clinical ranges of 15 - 75 ng ml^{-1} and 4 ng ml^{-1}, respectively. The sensitivity may be improved factors by addressing adverse factors such as denaturation of the proteins on the disc surface.

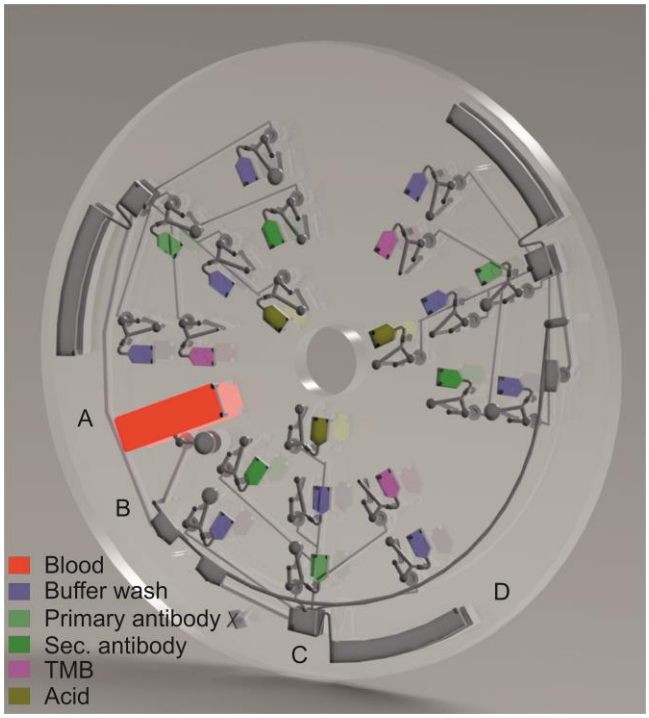

Figure 1: Illustration of a LoaD system for the detection of a prostate cancer panel composed of fPSA and Her2 markers from whole blood.

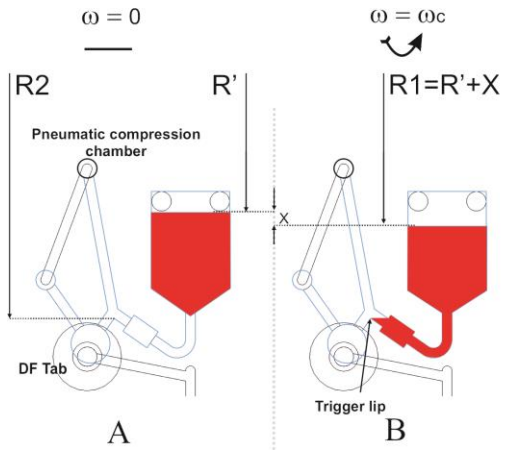

Figure 2: Actuation of centrifugo-pneumatically triggered DF valves based in the overflow lip mechanism. All distances are measured from the axis of rotation. (A) Initially, the liquid (red) is held back in the inlet reservoir. (B) At elevated spin rates, the centrifugally induced hydrostatic pressure compresses the enclosed gas so the liquid front advances past the trigger lip to open the DF.

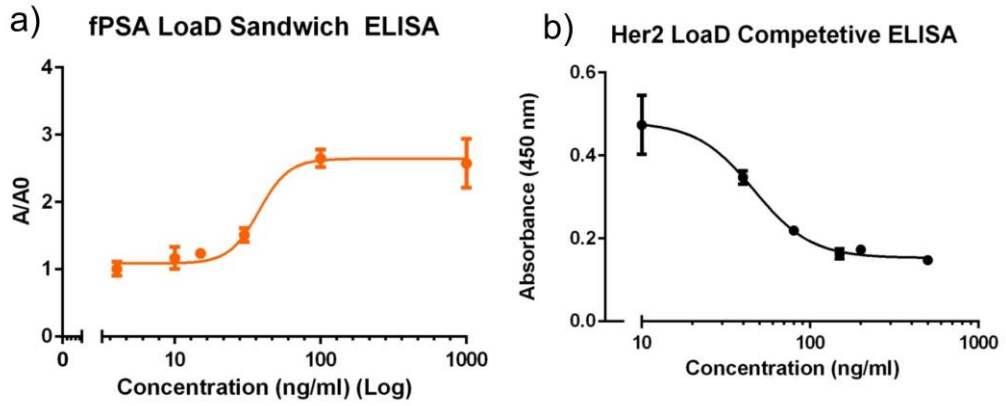

Figure 3: Signal-to-noise absorbance results for immunoassays on the LoaD to detect fPSA and Her2 biomarkers marker from buffer. Error bars indicate standard deviation from at least three measurements. The clinical ranges for the markers are: HER2 levels ranging from 15 ng ml^{-1} to as high as 75 ng ml^{-1} diagnosed as cancer. PSA level > 4.0 ng ml^{-1} is considered as a cut-off value for recommending biopsy [3].

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CONTACT

J. Ducrée. Ph: +353 (1) 700 5377. jens.ducree@dcu.ie