CENTRIFUGAL AUTOMATION OF A HIGHLY CUSTOMIZABLE RAPID-ELISA TEST TOWARDS DETECTION OF CERVICAL CANCER IN POINT-OF-CARE SETTINGS

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ABSTRACT

Infections of high-risk strains of human papilloma virus (HPV16-E7) have been directly linked with cervical carcinogenesis [1]. Despite pervasive HPV testing and vaccinations, resource-poor regions continue to be affected. We present here a fully automated, bead-based Rapid Antigenic Protein In-situ Display (RAPID) Enzyme Linked Immunosorbent Assay (ELISA) for the detection of the onco-proteins of HPV16 E7 [1]. Advanced centrifugal flow control is implemented by a novel router based on lipophilic membranes in an event-trigger valving architecture [2]. By simply replacing the charge of RAPID-functionalized beads in the incubation chamber, the system may be customized for the detection of other biomarkers.

KEYWORDS: Cervical cancer, Lipophilic membranes, Routing, RAPID ELISA, Centrifugal microfluidics, Lab-on-a-Disc (LoaD)

INTRODUCTION

Onco-proteins of HPV16 E7 virus have been shown to cause cervical lesions and to tackle this significant health issue, there is a dire need for point-of-care (POC) device for biomarker screening. The RAPID ELISA offers a customizable assay for the detection of this biomarker using functionalized magnetic beads. The automation of this complex multi-step assay is demonstrated on a LoaD that advances our previously developed, event-triggered centrifugal flow control [2] with a novel router based on lipophilic membranes.

EXPERIMENTAL

The entire sample handling protocol for the RAPID ELISA starting with plasma separation through to the reaction termination for absorbance measurement is fully integrated on a single LoaD device (Fig. 1). In order to avoid interference from the solid phase beads, lipophilic membrane facilitates the routing of the final reaction product from the incubation chamber to a distinct measurement chamber where the absorbance measurement can be made [3].

Anti-GST coated magnetic beads were pre-incubated with target antigen E7 that can then capture anti-HPV16-E7 Ab from a sample (while using anti-GST [26H1] as a control). On the LoaD platform, these beads were placed in the incubation chamber and held in place with a magnet wedged in the disc directly behind the chamber. An event-triggered [2] architecture the implements sequential release of sample and buffer / reagents washes over an incubation chamber with the stationary bead column.

RESULTS AND DISCUSSION

Figures 2 show the results for the detection of anti-HPV16-E7 antibody from buffer solution on a benchtop platform and the LoaD. The benchtop results in Fig. 2a results show a typical ELISA curve where the primary antibody is incubated for one hour (as compared to only 15 minutes on the disc). The LoaD results in Fig. 2b clearly show the ability to detect the target in the same concentration range. The total run time for the Load for the assay was 38 minutes compared to the total benchtop assay span of 138 minutes.

CONCLUSION

We demonstrate effective detection of the anti-HPV16-E7 antibody using an automated LoaD system. In clinical settings, the signals for HPV-specific Abs are measured as ratios of optical density (OD). These ratios are then compared to healthy controls to establish cutoff values [1].

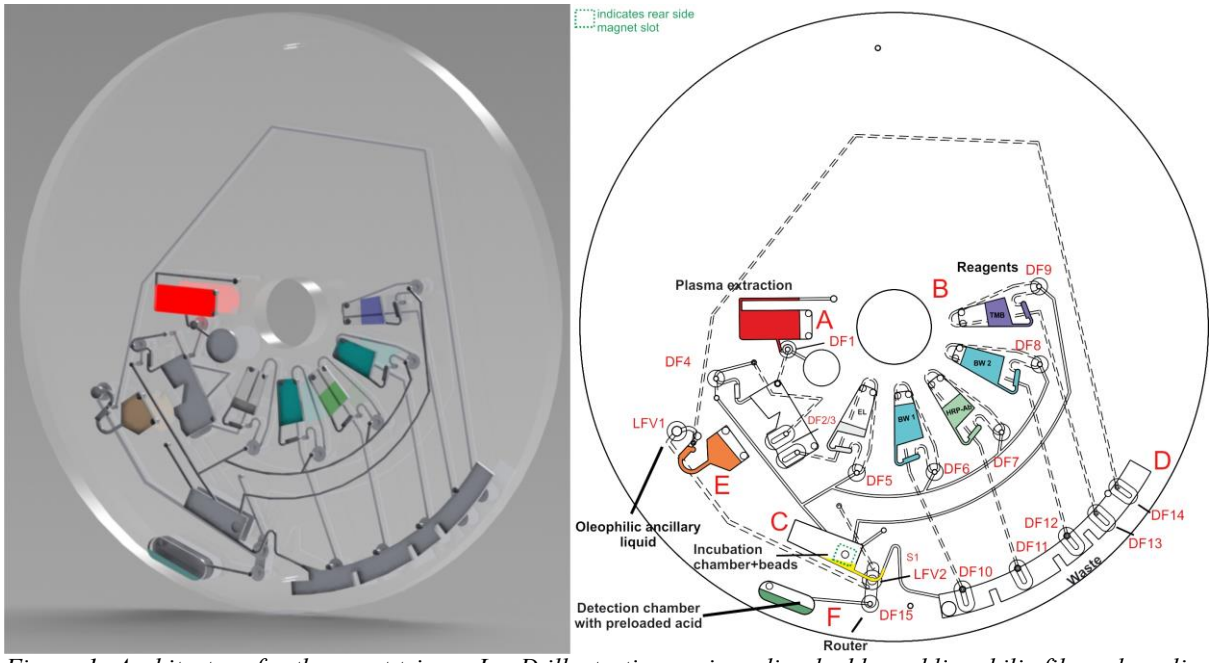

Figure 1: Architecture for the event trigger LoaD illustrating various dissolvable and lipophilic film valves, liquid chambers and pneumatic connections that allow the release the reagents in a cascade fashion. A- represents the plasma separation chamber where DF1 is released after the plasma is fully separated allowing the plasma to reach the incubation area C. B- represents the reagents and buffer washes that are released in a cascade fashion once the event triggers within the waste section D are activated by dissolution of the films. C- incubation chamber with a magnet at the back to hold the microspheres in place as a column. E- represents a routing structure with a high-pass valve for release of the routing fluid which eventually triggers the router mechanism in section F. The lipophilic films allow for controlled routing of the final reactant (TMB) to be moved away from the incubation chamber to a detection chamber with pre-loaded acid to quench the reaction and measure the absorbance without interference form the solid phase.

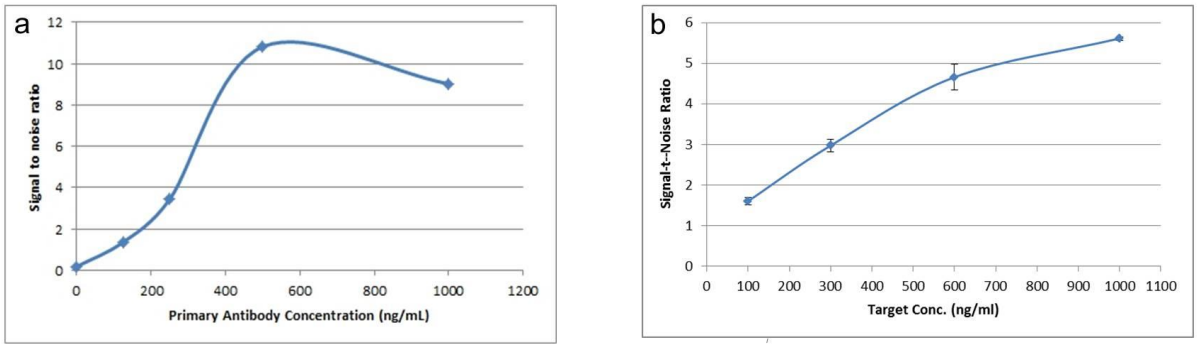

Figure 2: a) Detection of anti-HPV16-E7 from PBS buffer using RAPID ELISA protocol on a benchtop 96 well plate with magnetic beads coated with E7 antigen. Anti-GST [26H1] acts as a control which is performed with identical protocols to account for the interaction of the Primary Ab with the magnetic beads. The incubation of the Primary Ab is one hour (compared to the LoaD protocol where its 15 minutes). b) Detection of anti-HPV16-E7 from PBS buffer using RAPID ELISA protocol on the automated LoaD with magnetic beads coated with E7 antigen loaded in the incubation chamber. Anti-GST [26H1] acts as a control which is performed on separate disc with identical protocols. Error bars represent standard deviation from two measurements and two controls performed on separate discs.

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