Development of automated competitive ELISA paper-based analytical device using dissolvable sucrose valves for Aflatoxin B_1 detection

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Paper-based analytical devices are recognized as a powerful analytical tool for fluid handling and analysis. Achieving the goal of simple, inexpensive, user-friendly and on-site detection for paper-based device is especially difficult in competitive enzyme linked immunosorbent assay (ELISA) for low molecular weight compounds detection, which requires sequential delivery of reagents for sequential steps of reaction. Therefore, the device needs a special paper design to automate the fluid travel. In this study, a new paper-based device is proposed using a novel concept of dissolvable sucrose valves along with hydrophobic barriers fabricated by laser cutting machine. This method provides low cost, easy and quick device fabrication. It also prevents direct contact of reagents on the devices, resulting in automatic multistep sequencing of competitive ELISA. As a proof-of-concept demonstration for the method developed herein, the device is used to determine the levels of Aflatoxin B_1 (AFB_1), a carcinogenic agent.

For the designed pattern as shown in Figure 1, a multistep process of competitive ELISA is performed sequentially by a 3-steps; (1) the sample fluid laterally flows through the device, and allowing the competitive reaction happened between the sample and ALP-anti-AFB_1 mAb. Then, the flow directly passed through test zone while only excess ALP-anti-AFB_1 mAb can bind with immobilized antigen (AFB_1-BSA) at test zone, (2) the sucrose valves, that use for temporally reserve BCIP/NBT substrate, dissolve and then middle channel opens, (3) the BCIP/NBT substrate is then released to the test zone, resulting in a purple color from the enzymatic reaction.

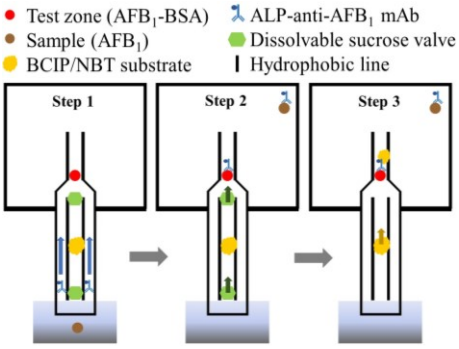

Figure 1. Schematic illustration of device

Due to competitive ELISA, the higher the sample antigen concentration, the weaker the eventual signal is reported as shown in Figure 2. From preliminary results, the device with dissolvable sucrose valves could be developed to detect AFB_1 in the concentration range of 0.14 ng mL^{-1} to 1,400 ng mL^{-1}. To increase the accuracy and precision of the device, the parameters of sucrose valves conditions and each component on the device e.g. size of device, width and length of barriers should be examined further.

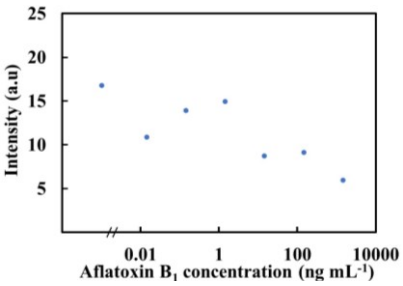

Figure 2. The detection of AFB_1